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METALION-DOPEDSILICON-SUBSTITUTEDCALCIUMPHOSPHATE BIOCERAMICS: PROGRESS IN BONE REGENERATION

LINRONG HE, RUOLAN LEI, #YU JIANG

Department of Gerontology, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, 646000, China

#E-mail: jiangyu2266@163.com

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The strategic modification of calcium phosphate bioceramics through silicon substitution and metal ion doping represents a significant advancement in bone tissue engineering. This review examines recent developments in the synthesis, characterisation, and biological performance of these advanced materials. Silicon incorporation fundamentally alters the physicochemical properties of calcium phosphates, enhancing their dissolution behaviour and surface reactivity. When combined with therapeutic metal ions, these materials demonstrate remarkable multifunctional properties, including enhanced osteogenic differentiation, improved angiogenic responses, and antimicrobial activity. The review discusses various doping mechanisms and processing techniques, highlighting how different synthetic routes affect ion incorporation efficiency and material properties. Particular attention is given to the complex relationships between the material composition, microstructure, and biological performance, including cell-material interactions and tissue response. Recent advances in characterisation methods have provided deeper insights into the mechanisms governing these materials' enhanced biological performance, while also revealing critical factors for their successful clinical implementation.

INTRODUCTION

Bone defects and injuries present significant challenges in orthopaedic and reconstructive surgery, with increasing prevalence occurring due to trauma, the ageing population, and pathological conditions [1]. The natural bone healing process, while remarkably efficient for minor injuries, often proves insufficient for critical-sized defects, necessitating therapeutic intervention [2]. This has driven extensive research into biomaterials that can effectively support and stimulate bone regeneration while maintaining mechanical integrity during the healing process. Calcium phosphate bioceramics have emerged as prominent materials for bone tissue engineering due to their chemical similarity to the mineral phase of natural bone [3, 4]. These materials, particularly hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), demonstrate excellent biocompatibility and osteoconductive properties. However, their clinical application often faces limitations related to the dissolution rates, mechanical properties, and biological activity [5]. To address these challenges, researchers have increasingly focused on modifying calcium phosphate bioceramics through ionic substitutions, with silicon incorporation emerging as a particularly promising strategy [6-8].

The incorporation of silicon into calcium phosphate structures represents a biomimetic approach, as silicon plays crucial roles in one's natural bone development and metabolism. Silicon substitution has been shown to enhance the biological performance of calcium phosphate (CaP) bioceramics through multiple mechanisms, including improved dissolution characteristics, increased surface reactivity, and enhanced protein adsorption [9-11]. These modifications result in materials that better support osteoblast adhesion, proliferation, and differentiation, ultimately leading to more effective bone regeneration. Building upon the benefits of silicon substitution, the strategic incorporation of therapeutic metal ions has emerged as an additional avenue for enhancing the functionality of these bioceramics [12, 13]. Metal ions, such as strontium, zinc, magnesium, and silver, can impart specific biological properties, including enhanced osteogenesis, improved angiogenesis, and antimicrobial activity [14, 15]. The combination of silicon substitution with metal ion doping represents a sophisticated approach to creating multifunctional biomaterials that can address multiple aspects of the bone healing process simultaneously.

The synergistic effects of silicon substitution and metal ion doping in calcium phosphate bioceramics have sparked considerable research interest over the past decade. These modifications can be achieved through various synthetic routes, each offering distinct

advantages in terms of ion incorporation efficiency, phase purity, and property control [16]. Understanding the complex relationships between the processing parameters, material properties, and biological performance is crucial for optimising these materials for clinical applications [17]. Recent advances in characterisation techniques and biological evaluation methods have provided deeper insights into the mechanisms by which silicon-substituted and metal ion-doped calcium phosphates influence cellular responses and tissue regeneration [18]. These findings have led to the development of increasingly sophisticated materials with carefully controlled composition, structure, and properties. However, several challenges remain in translating these materials from laboratory investigations to clinical applications, including questions about the long-term stability, optimal ion concentrations, and scale-up manufacturing processes.

This review aims to provide a comprehensive analysis of the current state of metal ion-doped silicon-substituted calcium phosphate bioceramics, with a particular focus on their applications in bone regeneration. This review examines the fundamental aspects of silicon substitution and metal ion incorporation, including their effects on material properties and biological performance. Special attention is given to the synergistic relationships between different ionic species and their impact on osteogenic, angiogenic, and antimicrobial properties. The review also explores recent developments in processing techniques, characterisation methods, and biological evaluation approaches. Moreover, it discusses emerging applications and future directions, including the development of composite materials, drug delivery systems, and surface modifications. Additionally, it addresses current challenges and limitations in the field, providing perspectives on potential solutions and future research directions.

REVIEW

Fundamentals of silicon-substituted calcium phosphate bioceramics

Crystal structure and phase composition

The crystal structure and phase behaviour of silicon-substituted calcium phosphates have been extensively studied due to their importance in biomedical applications. Silicon plays a crucial role in stabilising different calcium phosphate phases and can significantly influence the transformation temperatures between polymorphs. As demonstrated by Reid et al., single-phase silicon-substituted α-tricalcium phosphate (*α*-SiTCP) can be successfully synthesised through the sintering of silicon-enriched calcium phosphate precursors at temperatures around 1250 °C, with a silicon content ranging from 0.59 to 1.14 wt. % [19]. The incorporation of silicon into the crystal structure helps stabilise the *α*-TCP phase at lower temperatures compared to pure TCP. This is particularly significant since pure *α*-TCP is only stable at temperatures between 1430 – 1470 °C and requires rapid cooling to prevent phase transformation. As shown in Figure 1, the crystal structures of *α*- and *β*-TCP are distinctly different - *α*-TCP crystallises in the monoclinic space group P21/a, while *β*-TCP possesses a rhombohedral structure.

The phase composition and transformation behaviour of silicon-substituted calcium phosphates are heavily dependent on the silicon content and processing conditions. The study by Duncan et al. revealed that silicon substitution in *α*-TCP occurs primarily through the replacement of phosphate groups with silicate ions in specific crystallographic sites, resulting in significant changes to the unit cell parameters [20]. Through detailed X-ray diffraction and solid-state nuclear magnetic resonance analyses, they confirmed the presence of both silicate and disilicate species in the crystal structure, with silicon substitution causing a notable increase in the b-axis length and *β* angle. The stabilising effect of silicon on the *α*-TCP phase has been attributed to these structural modifications. Additionally, when synthesising hydroxyapatite (HA) with high silicon concentrations, an amorphous silicon-enriched phase tends to form first, which then transforms to *α*-SiTCP at lower temperatures compared to silicon-substituted HA (SiHA) [14]. This understanding of phase relationships and transformation mechanisms is crucial for controlling the final composition and properties of silicon-substituted calcium phosphate biomaterials.

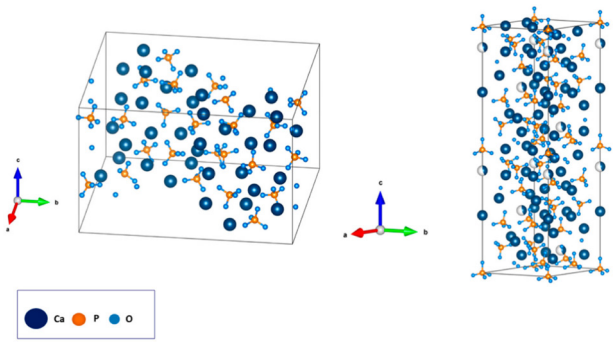


Figure 1. The crystalline structures of tricalcium phosphate (TCP): *α* form (left) and *β* form (right) [21].

Silicon incorporation mechanisms

Silicon incorporation into calcium phosphate bioceramics can be achieved through different methodological approaches, with each method offering distinct advantages for controlling the silicon content and release profile. Two main incorporation strategies - direct incorporation during crystal growth (CaP-I) and surface adsorption post-precipitation (CaP-A)

- lead to different structural and functional outcomes. The incorporation mechanism during crystal growth involves silicon ions becoming integrated into the crystal lattice of the CaP coating as it forms, potentially substituting for phosphate groups or occupying interstitial spaces. This was evidenced by the Energy--dispersive X-ray spectroscopy (EDS) analysis showing higher atomic percentages of silicon in the CaP-I samples compared to CaP-A, particularly at higher concentrations (17.9 % for CaP-I vs 7.97 % for CaP-A at 10 mM Si) [22]. The integration of silicon during crystal formation also appeared to influence the crystallisation process itself, as revealed by the scanning electron microscopy (SEM) analysis showing less sharp crystal morphology with the increasing silicon concentration. This structural modification suggests that silicon ions interfere with the normal crystal growth patterns of CaP, leading to smaller, less defined crystals [11].

The fundamental mechanisms governing the silicon release and biological effects appear to differ between the incorporation methods. As shown in Figure 2, the release profiles demonstrated that CaP-I coatings with 5 mM and 10 mM Si exhibited a more sustained release pattern over 14 days compared to the CaP-A coatings, where mainly the 10 mM condition showed substantial release. This difference in release kinetics likely stems from the distinct spatial distribution and binding of silicon within the coating structure. The incorporated silicon (CaP-I) appears to be more uniformly distributed throughout the coating matrix, allowing for the gradual dissolution as the CaP coating degrades. This was further supported by the biological response data, where CaP-I coatings generally showed higher alkaline phosphatase (ALP) activity and the more pronounced expression of osteogenic markers compared to the CaP-A coatings at equivalent silicon concentrations [22]. The surface-adsorbed silicon (CaP-A), while more readily available for initial release, may be more susceptible to rapid desorption, potentially explaining the more limited sustained effects observed in some biological assessments. These mechanistic differences highlight the importance of considering the incorporation method when designing silicon--substituted CaP biomaterials for specific therapeutic applications [23, 24].

Effect of silicon on physicochemical properties

The incorporation of silicon into calcium phosphate bioceramics significantly influences their physicochemical properties and biological performance. According to Porter [25], silicon-substituted HA exhibits relatively smaller grain size and less-ordered grain boundaries compared to stoichiometric hydroxyapatite, which directly affects its solubility and surface reactivity. This increased solubility plays a crucial role in accelerating the reprecipitation process around the implant surface and subsequent bone formation. The substitution mechanism typically involves silicate (SiO_4^{4-}) ions replacing phosphate (PO_4^{3-}) ions in the HA structure, with the negative charge being stabilised by hydroxide (OH^-) ion vacancy formation [26, 27]. As demonstrated by Botelho [28], increasing the silicon content in the HA lattice enhances bioactivity and leads to faster apatite formation. However, silicon substitution above 2 wt. % can destabilise the HA structure and promote the formation of α-tricalcium phosphate (α-TCP) [29].

The fundamental aspects of silicon-substituted calcium phosphate bioceramics are closely tied to their dissolution-reprecipitation behaviour and subsequent biological response. As shown in Figure 3, the morphological changes of osteoblastic cells on silicon-substituted HA ceramics demonstrate how the silicon content influences the cell behaviour and attachment. The figure clearly illustrates that moderate silicon substitution (0.8 wt. %) promotes optimal cell spreading and proliferation compared to both unsubstituted HA and higher silicon content (1.6 wt. %) [30]. This optimisation of cellular response is attributed to silicon's role in modifying the surface chemistry and topography of the bioceramics [31, 32]. The study by Pietak et al. [9] suggest that the release of aqueous silicon complexes to the extracellular matrix may be an additional factor promoting biological activity. The dissolution of silicon-substituted bioceramics accelerates the precipitation of a biologically equivalent HA layer, which serves as an ideal surface for protein adsorption and subsequent cell attachment [33]. This process is fundamental to the enhanced osteointegration and bone formation capabilities observed in silicon--substituted calcium phosphate bioceramics.

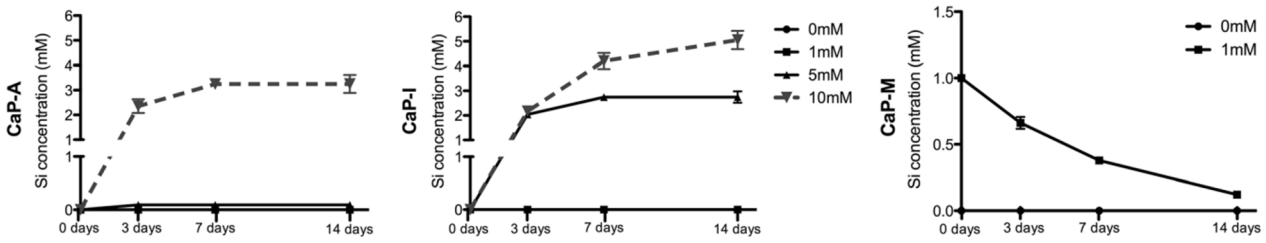


Figure 2. Elemental concentration of silicon in the cell culture medium after 3, 7 and 14 days of incubation in presence of CaP-A and CaP-I coatings made with 0, 1, 5 and 10 mM of Si and CaP-M coatings with 0 and 1 mM of Si [22].

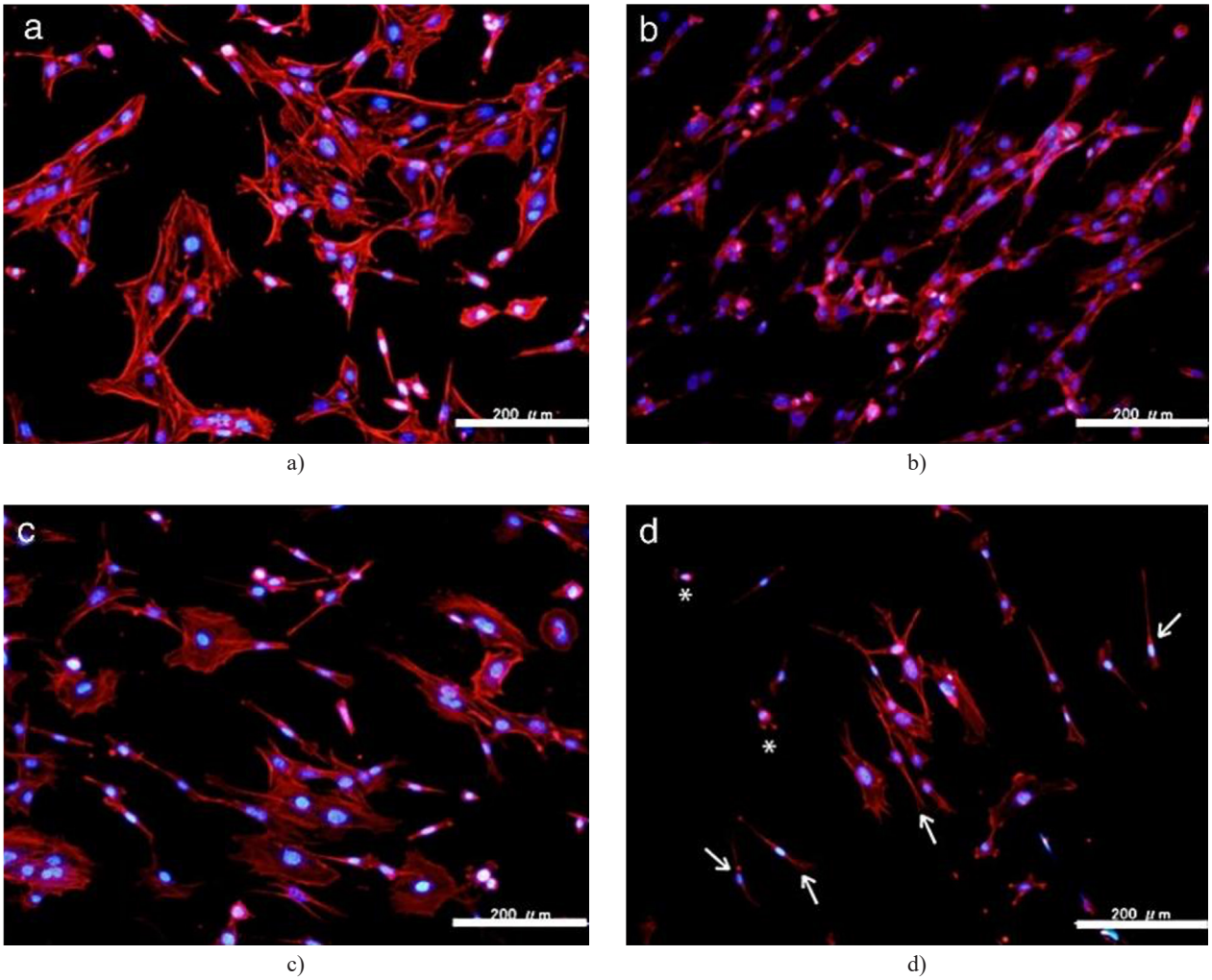


Figure 3. Morphological changes of the osteoblastic (MC3T3-E1) cells on Si substituted HA ceramics [13].

Influence on dissolution behaviour

The incorporation of silicon into HA structure significantly influences its dissolution behaviour, which directly impacts its bioactivity and clinical performance. Research has demonstrated that SiHA exhibits enhanced dissolution rates compared to pure HA, particularly at the grain boundaries and triple junctions. Porter et al. [34] observed that this increased dissolution is closely correlated with the silicon content, suggesting that the strategic incorporation of silicon can be used to control the material's degradation kinetics in physiological environments. The enhanced dissolution behaviour stems from multiple structural modifications induced by silicon substitution, including increased crystallographic defects, altered grain boundary chemistry, and modified surface properties. These modifications create preferential dissolution sites that facilitate the material's breakdown in biological environments while maintaining its overall structural integrity.

The dissolution mechanism of SiHA is fundamentally linked to its unique microstructural features and surface chemistry. Studies have shown that silicon incorporation leads to a significant decrease in the surface charge at a physiological pH (7.4), which promotes faster apatite formation on the material surface [35]. This effect is particularly pronounced when silicon is incorporated as tetrahedral silicate (SiO_4) groups rather than polymeric SiO_2 species, with optimal effects observed at silicon contents up to 1.2 wt. % [36]. The dissolution process is further enhanced by the presence of increased microstructural defects, particularly triple-junctions, which serve as initiation points for dissolution under in vivo conditions. Porter et al. demonstrated that these microstructural features contribute to the osteoconductive properties of SiHA while maintaining mechanical properties comparable to pure HA [37]. Importantly, the dissolution behaviour is not uniform throughout the material, but shows preferential patterns at the grain boundaries, which helps maintain the structural integrity of the implant while promoting beneficial biological responses. This controlled dissolution pattern is crucial for maintaining

the delicate balance between material degradation and new bone formation, ultimately contributing to the enhanced bioactive behaviour observed in SiHA compared to conventional HA implants [38].

Impact on mechanical properties

The incorporation of silicon into the HA structure significantly influences its mechanical and structural properties through several key mechanisms. As demonstrated through the X-ray diffraction (XRD) analyses, the silicon substitution leads to notable changes in the crystallinity and crystal size, which directly affect the material's mechanical behaviour. The substitution of PO_4^{3-} with SiO_4^{4-} results in smaller crystals with reduced crystallinity, primarily due to the larger ionic radius of Si^{4+} (0.042 nm) compared to P^{5+} (0.035 nm) [39, 40]. This structural modification is evidenced by the broadening of X-ray diffraction peaks and decreased peak intensities with increasing silicon content, as clearly illustrated in Figure 4. The figure demonstrates how higher silicon concentrations progressively impact the crystalline structure, showing a systematic reduction in the peak intensity from pure HA to $Si_{1.6}$HA, indicating the direct relationship between the silicon content and the structural modifications [39].

The mechanical implications of the silicon substitution extend beyond the crystallographic changes to influence the material's overall performance characteristics. Studies have shown that silicon incorporation results in slightly larger crystal lattice constants and an increased unit cell volume, which affects the material's mechanical stability and behaviour under load [41]. The structural refinement investigations conducted by Porter et al. [37] and El Yacoubi et al. [41] revealed that silicon incorporation causes a decrease in the a-axis and an increase in the c-axis of the HA crystal structure, creating unique mechanical properties that differ from conventional HA. These modifications lead to the formation of a highly hydrated surface layer surrounding the HA core crystals, which influences the material's interface with surrounding tissues and its mechanical integration capabilities [32, 42, 43]. The altered surface chemistry and morphology contribute to the enhanced bioactivity while maintaining mechanical stability, making silicon-substituted HA particularly suitable for load-bearing bone repair applications. Additionally, the presence of silicon has been shown to affect the sintering behaviour and thermal stability of the material, which are crucial factors in determining the final mechanical properties of the fabricated bioceramics [27].

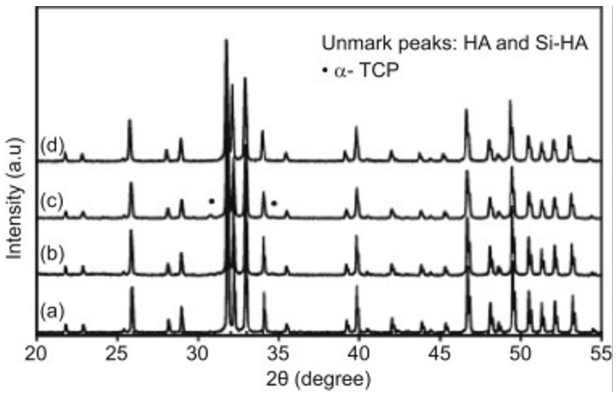


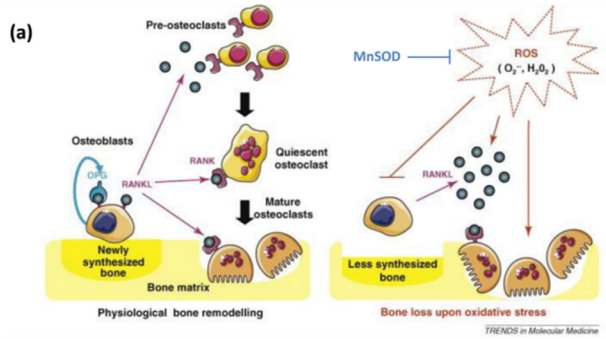
Figure 4. XRD patterns of the HA and silicon-substituted (Si-HA) powder after heat treatment at 1250 °C: (a) pure HA, (b) $Si_{0.4}$HA, (c) $Si_{0.8}$HA, and (d) $Si_{1.6}$HA [39].

Metal ion doping strategies and mechanism

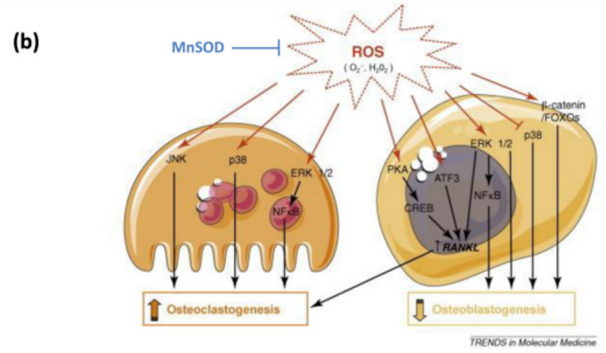
Selection criteria for therapeutic metal ions

The selection of appropriate therapeutic metal ions for doping CaP biomaterials requires the careful consideration of multiple factors, including their biological roles, concentration-dependent effects, and potential toxicity. A primary criterion is the ion's natural presence and established function in bone metabolism. Essential trace elements like zinc (Zn^{2+}), magnesium (Mg^{2+}), and manganese (Mn^{2+}) are integral components of bone tissue and play vital roles in various cellular processes related to bone formation and remodelling. For instance, Zn^{2+} is crucial for alkaline phosphatase activity and osteoblast function, while Mg^{2+} comprises about 0.65 % of the bone mineral content and influences both osteogenesis and angiogenesis [44]. Figure 5 illustrates how certain metal ions, such as manganese, can influence bone remodelling through their effects on reactive oxygen species (ROS) and the balance between osteoblast and osteoclast activity, demonstrating the complex biological mechanisms that must be considered in ion selection.

Another critical consideration is the controlled release profile and optimal therapeutic window of the selected ions. The paper emphasises that while many ions show beneficial effects at appropriate concentrations, they may become cytotoxic at higher levels. For example, copper (Cu^{2+}) and cobalt (Co^{2+}) ions can promote angiogenesis through vascular endothelial growth factor (VEGF) upregulation, but they also generate harmful reactive oxygen species at elevated concentrations that can damage cellular DNA [45, 46]. Therefore, the selection process must carefully evaluate the ion's concentration-dependent effects and establish safe dosage ranges. Additionally, the ionic radius and charge of the selected metal ions influence their ability to substitute into the CaP crystal structure, affecting material properties and release kinetics. As noted in the review, strontium (Sr^{2+}) with



a)



b)

Figure 5. a) schematic demonstrating the normal bone remodelling process (left) between osteoblasts and osteoclasts and b) A more detailed look at the signalling pathways ROS has been shown to influence in both osteoclasts and osteoblasts [47].

an ionic radius of 1.13 Å leads to lattice expansion, while magnesium (Mg^{2+}) at 0.69 Å causes lattice contraction, impacting the material's stability and degradation characteristics.

Doping methods and processing techniques

The incorporation of metallic dopants into CaP materials can be achieved through various synthesis and processing routes, with the choice of method significantly impacting the final properties and performance. The substitution mechanism primarily depends on the ionic radius of the dopant compared to calcium (Ca^{2+}, 0.99 Å) - for example, strontium (Sr^{2+}, 1.13 Å) increases the unit cell size when substituting for calcium, while magnesium (Mg^{2+}, 0.69 Å) and zinc (Zn^{2+}, 0.74 Å) cause lattice contraction [48]. Silicon doping typically involves Si^{4+} substituting for phosphate (P^{5+}) groups, which introduces crystal defects and increases the material solubility [9]. The processing parameters, particularly sintering temperature and atmosphere, must be carefully controlled as they affect the phase stability, densification behaviour, and the final distribution of dopants within the CaP structure.

The processing techniques for producing doped CaP materials have evolved to include both conventional and advanced manufacturing methods. Traditional approaches like wet chemical precipitation and solid-state reactions remain widely used, while newer techniques such as sol-gel processing and three-dimensional printing enable greater control over composition and architecture [49]. When multiple dopants are incorporated simultaneously, their interactions can affect the substitution behaviour and material properties - for instance, the presence of one dopant may influence the substitution mechanism of another [50]. The concentration of dopants must be carefully optimised, as excess amounts can destabilise the crystal structure or lead to the formation of secondary phases. Heat treatment conditions also play a crucial role, as different dopants can affect the temperature stability of various CaP phases - for example, magnesium helps stabilise the β-phase of tricalcium phosphate at higher temperatures by delaying the β to α phase transformation [51].

Ion substitution mechanisms

The ion substitution mechanisms in CaP materials are primarily governed by the ionic size, charge balance, and crystal structure compatibility of the dopant species. For divalent cations like Sr^{2+}, Mg^{2+}, and Zn^{2+}, the substitution typically occurs at Ca^{2+} sites in the crystal lattice. The ionic radius plays a crucial role in determining the structural effects - Sr^{2+} (1.13 Å) causes lattice expansion due to its larger size compared to Ca^{2+} (0.99 Å), while smaller ions like Mg^{2+} (0.69 Å) and Zn^{2+} (0.74 Å) lead to lattice contraction [48]. These size differences affect not only the crystal structure, but also influence material properties such as solubility, thermal stability, and biological response. Silicon incorporation follows a different mechanism, where Si^{4+} typically substitutes for P^{5+} groups, requiring charge compensation mechanisms that can create oxygen vacancies or other structural defects [9, 31].

The complexity of ion substitution increases significantly when multiple dopants are incorporated simultaneously, as their interactions can affect both the substitution mechanisms and resulting material properties. The presence of one dopant can influence the substitution behaviour of another through changes in the local charge distribution and crystal field effects [50]. For instance, when magnesium is present along with silicon in β-tricalcium phosphate, it can affect the phase stability and transformation temperatures differently than either dopant alone. The substitution mechanisms also impact the material's biological performance through various pathways - for example, strontium substitution has been shown to activate calcium-sensing receptors (CaSRs) in osteoblasts, leading to enhanced bone formation [52]. The release kinetics of these substituted ions are controlled by the strength of ionic bonds and the local crystal environment, which, in turn, affects their biological availability and therapeutic efficacy [53, 54].

Control of ion release kinetics

The control of ion release kinetics in metal-doped CaP materials is crucial for achieving optimal therapeutic effects in bone tissue engineering applications. Unlike surface-loaded pharmacologics and biologics that exhibit burst release patterns, properly incorporated dopant ions can provide sustained release over extended periods. As shown in Figure 6, the incorporation of different dopant combinations (MgO, SrO, and SiO_2) into TCP significantly affects the degradation behaviour and mechanical properties over time in simulated body fluids (SBFs). The figure demonstrates that doped TCP samples exhibit more controlled and gradual strength degradation compared to pure TCP, indicating that dopant incorporation can effectively modulate the dissolution rates and ion release profiles [50]. This controlled release is particularly important for maintaining therapeutic ion concentrations within the optimal range for stimulating osteogenesis and angiogenesis while avoiding potential cytotoxic effects that could result from burst release.

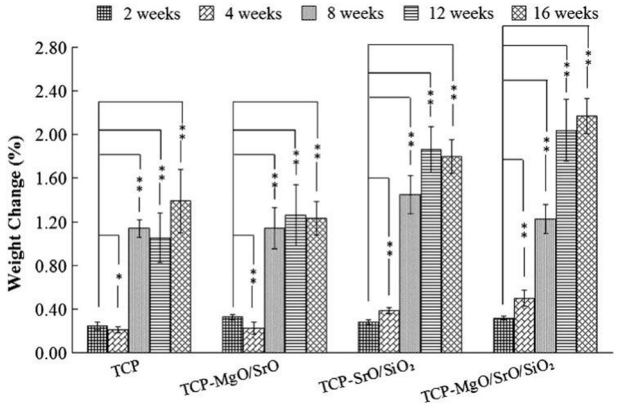


Figure 6. Weight change as a function of time for TCP, TCP-MgO/SrO, TCP-SrO/SiO_2 and TCP-MgO/SrO/SiO_2 in the simulated body fluid [50].

The release kinetics of dopant ions are influenced by multiple factors, including the substitution mechanism, crystal structure, and local chemical environment within the CaP matrix. When dopants are incorporated through ionic substitution rather than surface modification, their release is typically coupled with the material's degradation rate and can be controlled through the composition optimisation and processing parameters. For instance, the presence of silicon in the CaP structure can increase material solubility through the creation of crystal defects, thereby affecting the release rates of co-substituted ions [9]. The combination of different dopants can create synergistic effects on release profiles - for example, the co-substitution of strontium and magnesium has been shown to provide more desirable degradation characteristics compared to single--dopant systems, leading to enhanced biological response in vivo [50, 53]. Understanding and controlling these release mechanisms is essential for developing next--generation biomaterials that can provide therapeutic ion concentrations over clinically relevant timeframes while maintaining structural integrity during the bone healing process.

Biological performance

Osteogenic differentiation and bone formation

Silicon doping has emerged as a promising strategy for enhancing the biological properties of biphasic calcium phosphate (BCP) scaffolds for bone tissue engineering. As demonstrated by Lu et al. [55], the incorporation of silicon ions into the BCP structure significantly influences both its physicochemical properties and cellular responses. Their research revealed that silicon doping levels between 2 – 4 mol. % optimally promoted the osteogenic differentiation of mesenchymal stem cells, with 4 mol. % showing the most favourable outcomes. This was evidenced by the enhanced expression of the osteogenic markers including Runx2, Col-I, ALP, OPN, BSP, and OCN (Figure 7). However, they found that excessive silicon doping (≥ 6 mol. %) had inhibitory effects on the stem cell differentiation, highlighting the importance of controlled silicon incorporation.

The *in vivo* validation studies further confirmed the effectiveness of silicon-doped BCP scaffolds in promoting bone regeneration. When implanted in rabbit femoral defects, BCP scaffolds containing 4 mol. % silicon (Si_4-S) demonstrated superior bone formation compared to the non-doped scaffolds (Si_0-S) and blank controls. As shown in Figure 9, the micro--CT and histological analyses revealed that Si_4-S scaffolds achieved approximately 30 % new bone formation after 12 weeks, significantly higher than Si_0-S (~23 %) and the blank controls (~10 %). This enhanced bone regeneration was attributed to the optimal silicon release kinetics and degradation profile of the Si_4-S scaffolds, which created a favourable microenvironment for osteogenic differentiation while maintaining adequate mechanical properties [4]. These findings align with previous studies showing silicon's important role in bone tissue formation and mineralisation [56, 57], making silicon-doped BCP scaffolds promising candidates for clinical bone defect repair applications.

Angiogenic responses

The incorporation of metallic elements into CaP bioceramics has emerged as a promising strategy for enhancing both osteogenic and angiogenic properties critical for bone tissue engineering. Silicon, as a metalloid element, plays a fundamental role in bone mineralisation and demonstrates significant proangiogenic capabilities when incorporated into CaP structures. The mechanism behind Si-induced angiogenesis involves the upregulation

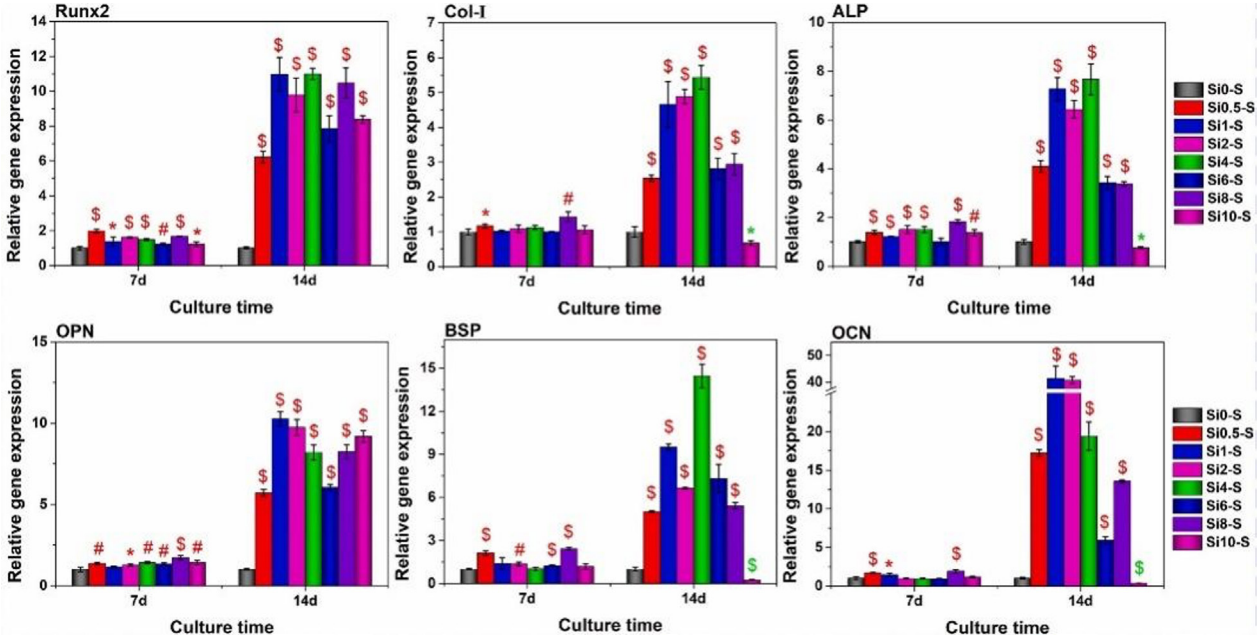


Figure 7. Osteogenesis-related genes expression [58].

of endothelial nitric oxide synthase (eNOS), which leads to the increased production of crucial proangiogenic growth factors including VEGF, bFGF, and TGF-β. Additionally, silicate ions can modulate the cellular calcium levels, directly affecting the proliferation and motility of vascular endothelial cells, thereby promoting the formation of new blood vessels. As highlighted in the research, Si-doped CaP materials have demonstrated superior biological properties compared to their unmodified counterparts, particularly in terms of tissue repair and regeneration applications [59].

The synergistic effects of combining multiple metallic dopants have also shown promising results in enhancing the angiogenic responses. The co-doping of silicon with other elements, such as zinc, copper, and cobalt, can create more potent angiogenic biomaterials. For instance, Cu^{2+} and Co^{2+} ions have demonstrated the ability to activate specific signalling pathways, including the hypoxia-inducible HIF-1 and mitogen-activated protein kinase (MAPK) pathways, which are crucial for blood vessel formation [60]. The research indicates that the careful control of dopant concentrations is essential, as excessive levels of certain elements, particularly cobalt, may lead to adverse effects on living systems. This delicate balance highlights the importance of optimising the composition and release kinetics of metal-doped CaP materials to achieve optimal therapeutic outcomes in bone tissue engineering applications [61].

Antibacterial properties

Metal-doped calcium silicate cements, particularly those incorporating zinc, have emerged as promising materials for bone filling applications due to their dual functionality in promoting bone regeneration while providing antimicrobial protection. The incorporation of zinc ions into the calcium silicate framework results in sustained antimicrobial activity through multiple mechanisms. As shown in Figure 8, zinc-doped tricalcium silicate demonstrates significant antibacterial effects against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, with an effectiveness observed at concentrations as low as 30 $\mu g \cdot ml^{-1}$ after 24 hours of exposure. This is notably more efficient than conventional zinc oxide, which typically requires 300 – 600 $\mu g \cdot ml^{-1}$ to achieve similar effects [62]. The enhanced antibacterial activity stems from the synergistic action of zinc and calcium ions, where the hydration of tricalcium silicate produces calcium hydroxide and zinc hydroxide, maintaining a stable alkaline pH of 11 – 12 in the microenvironment [63].

The mechanistic pathway of bacterial inhibition involves multiple cellular targets. When bacteria encounter the zinc-doped cement, the released zinc ions accumulate at the bacterial membrane, leading to disruption of membrane integrity and cellular function. This is particularly effective against Gram-negative bacteria like *E. coli*, which possess an additional outer membrane layer of negatively charged lipopolysaccharides that attract the positively charged zinc and calcium ions. As demonstrated through the scanning electron microscopy analysis, this interaction results in visible membrane damage, including the loss of the typical bacterial morphology and cytoplasmic leakage [64]. The presence of calcium hydroxide further enhances the antibacterial effect by creating an unfavourable alkaline environment that inhibits

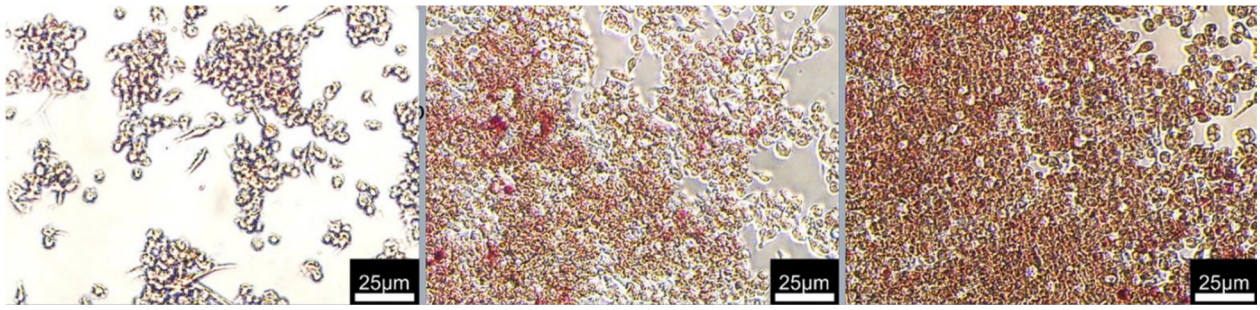


Figure 8. TRAP staining of osteoclasts differentiated from mice macrophages (RAW264.7), in the presence of RANKL [66].

the bacterial enzymatic activity and cellular division. The sustained release of zinc ions from the cement matrix, as shown through ion release studies, ensures a prolonged antimicrobial protection, making these materials particularly suitable for clinical applications where long-term infection prevention is crucial [65].

Cell-material interactions

Metal-doped calcium phosphate materials exhibit unique cell-material interactions that can significantly influence the cellular responses and tissue regeneration outcomes. As shown in Figure 9, cobalt-doped hydroxyapatite nanoparticles demonstrate a distinctive interaction with the bone tissue, promoting regeneration of the osteoporotic alveolar bone despite showing some cytotoxicity towards bone cells in vitro. This dual nature of metal-doped calcium phosphates highlights the complex relationship between the material composition and the biological response [67]. The incorporation of various metallic ions such as $Fe^{2+}/^{3+}$, $Co^{2+}/^{3+}$, and Gd^{3+} into the calcium phosphate structure can impart magnetic properties, while rare earth elements like Eu^{3+}, Yb^{3+}, and Tb^{3+} contribute photoluminescent characteristics that enable advanced imaging capabilities [68, 69]. These functionalities arise from the remarkable ion-exchange properties of calcium phosphates, which can accommodate more than half of the periodic table's elements within their crystal lattice structure [70].

The interaction between cells and metal-doped calcium phosphates is highly dependent on the specific ionic substitutions and their concentrations. For instance, selenium-doped hydroxyapatite demonstrates enhanced antibacterial properties that increase proportionally with the selenite content, while simultaneously maintaining the ability to promote osteogenic responses [71-73]. Similarly, europium-doped hydroxyapatite exhibits multicolour luminescence under visible light excitation and maintains stability up to 15 weight percent of Eu^{3+} incorporation, enabling effective cell imaging applications. The versatility of these materials is further demonstrated through the synergistic effects observed when multiple ionic species are incorporated simultaneously, such as in the case of silver and lanthanide co-doping, which combines antimicrobial and spectroscopic properties [74]. These interactions are particularly relevant for bone tissue engineering applications, where the careful selection of dopant ions can help achieve desired therapeutic outcomes while maintaining biocompatibility and promoting proper tissue regeneration [67, 75].

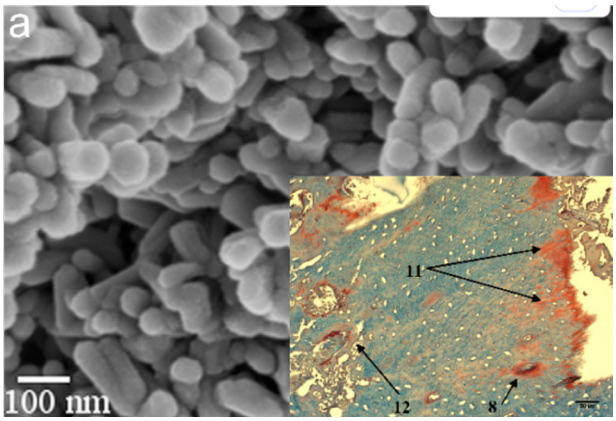


Figure 9. SEM image of hydroxyapatite nanoparticles in which Ca^{2+} ions were partially substituted with Co^{2+} ions. The inlet shows an almost complete resorption of the implant and the regeneration of an osteoporotic bone 24 weeks after implantation [67].

CONCLUSIONS

Metal ion-doped silicon-substituted calcium phosphate bioceramics have demonstrated remarkable potential in bone regeneration applications through their enhanced biological performance and versatile functionality. The incorporation of silicon has been shown to significantly improve the materials' bioactivity through multiple mechanisms, including enhanced dissolution characteristics, increased surface reactivity, and improved protein adsorption. The strategic incorporation of therapeutic metal ions such as strontium, zinc, magnesium, and silver provides additional benefits, including enhanced osteogenesis, improved angiogenesis, and antimicrobial properties. The synergistic effects between the silicon substitution and metal ion doping have resulted in materials with

superior performance compared to conventional calcium phosphates, particularly in terms of controlled ion release and cellular responses. Studies have demonstrated that silicon substitution levels between 2 – 4 mol. % optimally promote the osteogenic differentiation of mesenchymal stem cells, while the incorporation of zinc ions provides sustained antimicrobial activity through multiple mechanisms. The research has also revealed that silicon--doped materials exhibit enhanced dissolution rates at the grain boundaries and triple junctions, which contributes to their improved bioactive behaviour while maintaining structural integrity.

Despite these significant advances, several challenges remain in translating these materials from laboratory investigations to clinical applications. The complexity of manufacturing processes and the need for precise control over the composition and structure present significant scalability challenges. The optimisation of ion release kinetics and degradation rates continues to be crucial for achieving desired therapeutic effects while maintaining structural integrity during the bone healing process. Additionally, questions about the long-term stability, optimal ion concentrations, and scale-up manufacturing processes need to be addressed. Nevertheless, the demonstrated ability of these materials to promote both bone formation and vascularisation while providing antimicrobial protection represents a significant advantage for clinical applications. The field holds considerable promise for developing personalised treatment approaches through tailored composition and properties, particularly for challenging bone regeneration scenarios where conventional treatments show limited success. Continued research and development efforts, coupled with advancing manufacturing technologies, suggest a bright future for their application in regenerative medicine.

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