Biomaterials Science

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Biomater. Sci.*, 2023, **11**, 7003

Recent advances in the application and biological mechanism of silicon nitride osteogenic properties: a review

Ziyi Liu,† Ruijie Wang,† Wenjing Liu, Yushan Liu, Xiaoli Feng, Fujian Zhao, Pei Chen, Longquan Shao * and Mingdeng Rong *

Silicon nitride, an emerging bioceramic material, is highly sought after in the biomedical industry due to its osteogenesis-promoting properties, which are a result of its unique surface chemistry and excellent mechanical properties. Currently, it is used in clinics as an orthopedic implant material. The osteogenesis-promoting properties of silicon nitride are manifested in its contribution to the formation of a local osteogenic microenvironment, wherein silicon nitride and its hydrolysis products influence osteogenesis by modulating the biological behaviors of the constituents of the osteogenic microenvironment. In particular, silicon nitride regulates redox signaling, cellular autophagy, glycolysis, and bone mineralization in cells involved in bone formation *via* several mechanisms. Moreover, it may also promote osteogenesis by influencing immune regulation and angiogenesis. In addition, the wettability, surface morphology, and charge of silicon nitride play crucial roles in regulating its osteogenesis-promoting properties. However, as a bioceramic material, the molding process of silicon nitride needs to be optimized, and its osteogenic mechanism must be further investigated. Herein, we summarize the impact of the molding process of silicon nitride on its osteogenic properties and clinical applications. In addition, the mechanisms of silicon nitride in promoting osteogenesis are discussed, followed by a summary of the current gaps in silicon nitride mechanism research. This review, therefore, aims to provide novel ideas for the future development and applications of silicon nitride.

Received 20th May 2023,
Accepted 5th September 2023

DOI: 10.1039/d3bm00877k

rsc.li/biomaterials-science

1. Introduction

It is difficult for bone tissue defects caused by surgery, injury, tumor, and other reasons to be restored to their original state by self-repair.[1] The healing and regeneration of bone tissue is a vital and challenging part of clinical treatment. The traditional approach is constrained by the limited autologous bone availability, while allogeneic bone grafting is constrained by immune rejection. Bone tissue regeneration engineering provides a more promising way for bone tissue healing and regeneration, and has gradually become a key focus of modern medical treatment.[1,2] Biocompatible materials with autologous bone-replacement and osteogenesis-promoting effects such as bioceramics, bioglass and metallic materials are gradually being discovered and used in clinical settings to accelerate bone healing. Nevertheless, they still have limitations in some aspects of application. For example, bioglass and calcium phosphate ceramics have limitations such as low antibacterial properties (hence the need for additional anti-infection means for postoperative infection prevention and control), as well as brittleness.[3,4] The limitations of metallic materials are highlighted by oxidation/corrosion after exposure to body fluids,[5] aesthetics and imaging examination artifacts.[6,7] Another bioceramic, silicon nitride, has strong comprehensive properties, including good physicochemical, antibacterial, and osteogenic properties.

Silicon nitride (Si_3N_4) is a non-oxide ceramic extensively used in metal processing, bearing manufacturing, high-temperature engine component fabrication, and the aerospace industry because of its excellent mechanical properties, including high-temperature stability, thermal-shock resistance, high stiffness, high hardness, wear resistance, and fracture resistance.[8] Silicon nitride has received great attention in recent years because of its sound biological properties, in addition to excellent physicochemical and mechanical properties. Several *in vivo* and *in vitro* studies have revealed that silicon nitride significantly enhances the antimicrobial properties and osteoinductive ability of the environment surrounding the material.[9–12] The key to successful orthopedic and dental

Stomatological Hospital, Southern Medical University, Jiangnan Avenue 366, Guangzhou 510280, China. E-mail: shaolongquan@smu.edu.cn, rmdeng@smu.edu.cn

†These authors contribute equally to this work.

implant placement is the successful healing of the peri-implant bone tissue, and the degree of control of peri-implant infection and rate of bone tissue production are among the decisive influencing factors.[13,14] Owing to the aforementioned excellent biological and physicochemical properties, silicon nitride is considered a promising material for bone regeneration engineering and is currently used in this way. Its applications primarily include joint replacement[15] and spinal spacer.[16,17]

The unique biological properties of silicon nitride stem from its surface chemistry. Similar to other silicon compounds, silicon nitride undergoes a nucleophilic substitution reaction (SN2) in an aqueous solution, where water acts as a nucleophile attacking the silicon atom, the Si–N bond breaks, the N vacancy attracts protons dissociated from the water molecule to form NH_3 and NH_4^+, and the Si vacancy is replaced by OH^- to form surface silanols (Si-OH), as well as orthosilicic acid ($Si(OH)_4$ or H_4SiO_4), which is water soluble.[18,19] The reactions can be expressed as follows:

$$Si_3N_4 + 6H_2O \rightarrow 4NH_3 + 3SiO_2, \quad (1)$$

$$SiO_2 + 2H_2O \rightarrow Si(OH)_4, \quad (2)$$

$$NH_3 + H_2O \rightleftharpoons OH^- + NH_4^+ \quad (3)$$

On the one hand, Si is primarily present in solution as orthosilicate [H_4SiO_4 or $Si(OH)_4$], which is a soluble form.[20] However, oxidation converts a portion of the silicon on the surface of silicon nitride to SiO_2. Since the dissociated product of SiO_2 and silicate are identical in aqueous solution, therefore, additional silicon-containing ionic groups in the aqueous solution surrounding silicon nitride must be present (hereafter referred to as Si ions (Si^{4+}) for convenience).[21,22] Studies have demonstrated that silicon has good osteogenic activity,[23,24] and that orthosilicic acid, the only soluble form of silicon in an aqueous solution, can influence a cell's biological behavior intracellularly by modulating various signal transductions, which eventually promotes osteogenesis.[25,26] On the other hand, the N element is present primarily as NH_4^+ in a low pH and NH_3 in a high pH.[20] Studies have shown that NH_4^+ is involved in the metabolic pathway of glutamine synthetase formation, while glutamine provides the material synthesis raw material for osteogenesis-related cell differentiation and proliferation.[9] Besides, with the involvement of cells, the silicon nitride surface group silanol can undergo a series of cascade reactions with ammonia to produce biologically active substances with bidirectional effects – reactive nitrogen species (RNS).[9,27] RNS further regulate signal pathways associated with cell metabolism and differentiation by activating redox signals in osteoblast cell lines, thereby regulating the rate of bone tissue regeneration.[27] In addition, these substances have the potential to affect immune cells and vascular endothelial cells, which also play a crucial regulatory role in the osteogenic microenvironment (see in Fig. 1).

There is a lack of systematic studies on the osteogenesis-promoting mechanism of silicon nitride, and the applications

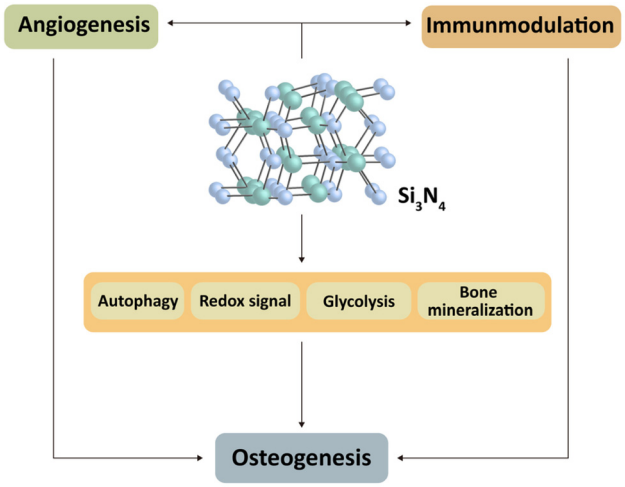

Fig. 1 Overview of mechanism of osteogenesis properties of silicon nitride.

and mechanisms of silicon nitride in bone tissue regeneration have not been summarized. In this paper, we review the current applications of silicon nitride in bone tissue engineering and other potential applications in the future, with a focus on the effects of silicon nitride in bone regeneration microenvironments. Moreover, we discuss the mechanisms behind its effects on the biological behavior of a cell. Furthermore, the factors affecting the performance of silicon nitride in promoting osteogenesis are addressed with the goal of providing theoretical guidance for future research and the development of applications for silicon nitride's osteogenesis-promoting properties.

2. Application of silicon nitride osteogenic properties

Silicon nitride has been shown to have good biocompatibility and promote bone formation.[28–31] Correspondingly, it has been used as an artificial bone graft or bone implant material. However, the clinical application of silicon nitride is still in its infancy. The following section describes the silicon nitride molding process and its effects on the properties of silicon nitride, the benefits and applications of nano-scale silicon nitride, as well as the existing and potential applications in clinical orthopedics and dentistry.

2.1 Molding process of silicon nitride implants and its impact on performance

When used to create artificial bone or implant materials, silicon nitride faces numerous issues, such as high brittleness, high porosity, and insufficient strength as a bioceramic material. The properties of bioceramic implants frequently depend on the properties of the material. In addition, different factors involved during the molding methods of the material significantly impact the properties of the final

product,[32] as does silicon nitride. There are three crystallographic structures of silicon nitride: α, β and γ phase. And the first two are the more common form of existence. The hardness of α-Si_3N_4 is greater than β-Si_3N_4, while β-Si_3N_4 possesses more stable chemical properties.[33] As the temperature increases, α phase will gradually transform into β phase. The molding processes discussed below all undergo high temperature sintering, so the finished Si_3N_4 is generally dominated by β phase even if manufactured by α-Si_3N_4 powder.

Reaction bonding, sintering, and pressure-assisted sintering are the three traditional techniques for producing bulk silicon nitride (porous and dense) with specific shapes. For reaction bonding, nitrogen at a high temperature is used to heat porous silicon blocks with specific geometries formed from silicon powder. The sintering method refers to the compaction of silicon nitride powder (combined with sintering agent Al_2O_3, Y_2O_3), followed by heating in high-temperature N_2 (20–30 MPa). Based on the sintering method, hot pressing technology can produce more dense silicon nitride than sintering due to the higher given sintering pressure.[34,35] The traditional method of silicon nitride molding described previously is more complicated. Because of its rapid molding speed and low cost, three dimension printing (3D printing) has been increasingly used in the fabrication of ceramic products.[36] However, due to the increase in internal stress caused by rapid cooling after printing, direct 3D printing often results in cracks in the final product obtained. Indirect 3D printing technology comprises primary binding followed by sintering, thereby combining 3D printing technology with traditional techniques to prevent cracks that can result from excessive internal stresses on products of different volume sizes.[37–40] Correspondingly, this method has now been applied to the preparation of bulk silicon nitride.[41–43] Silicon nitride scaffolds produced through indirect 3D printing exhibited high levels of surface biological activity, protein adsorption, and osteogenic activity.[44] There are several 3D printing technologies used to fabricate silicon nitride: fused deposition modelling (FDM), laminated object manufacturing (LOM), binder jetting (BJ) and digital light processing (DLP).[39,45] The mechanical properties of silicon nitride prepared by FDM and LOM are comparable to those of silicon nitride prepared by reaction bonding, casting and sintering.[45] However, a disadvantage of LOM is its limited capability to produce parts with internal cavities,[46] as well as poor surface quality of FDM product makes them unsuitable for the fabrication of artificial bone implants with high precision.[45] BJ and DLP have relatively higher accuracy than the first two technologies.[45] In this regard, the binder jetting process employs epoxy binder to produce products with a porosity range of 30–40% and a bending strength of 53 ± 2 MPa. The digital optical processing (DLP)-based finished product had a bending strength of only 1 MPa and a yield strength of 43.28.[39] BJ and DLP fabricated silicon nitride products are also plagued by defects, such as an excessively high porosity,[47] thereby resulting in poor mechanical properties. The defects of mechanical properties of silicon nitride produced by DLP are caused by poor light transmittance of silicon nitride powder and large refractive index difference between silicon nitride powder and resin, which lead to insufficient light curing depth. Products fabricated by BJ and DLP require to be further densified. Amorphous oxide film is formed on the surface of silicon nitride powder after it is oxidized at 1150–1200 °C for 1–3 h. Using the treated powder as raw material for DLP, the curing depth also increased with the oxide layer incrassating, and the relative density of the final product reached 90%.[48] After precursor infiltration and pyrolysis, the porosity of DLP-fabricated silicon nitride products decreased significantly, while the mechanical properties increased (porosity jaw bending strength was 1.30% and 162.35 MPa, respectively).[40] Surface oxidation and PIP may offer a viable solution to the problem of high porosity and poor mechanical properties of silicon nitride products. Moreover, the proportion of printing paste of BJ and DLP also needs to be optimized. To summarize, the fabrication of medically-dense silicon nitride production may be dominated by indirect 3D printing in the future. Additionally, resolving issues such as insufficient porosity and mechanical properties of 3D-printed silicon nitride is likely to become the primary research focus of future silicon nitride molding techniques.

In the context of bone implants, the porosity of silicon nitride implants must be carefully considered. It has been noted that the reduction of porosity in silicon nitride is frequently accompanied by an increase in mechanical properties.[49] However, the porous structure enables greater cell adhesion on the surface of silicon nitride, which maximizes the use of bioactive ions produced on the surface and promotes the rapid formation of an osteogenic microenvironment.[50,51] In various applications, the porosity and mechanical properties of silicon nitride are subject to distinct specifications. For instance, when silicon nitride is used as a scaffold material for bone engineering, it must ensure that the material has a certain porosity and a certain strength while forming a porous structure so that the silicon nitride scaffold can maintain its initial form and perform its function of guiding bone remodeling.[32,52] For bone implant materials such as the intraosseous portion of an artificial joint and dental implants, silicon nitride must possess a particular density to ensure the material's strength. How to construct a material's bioactive surface is an issue that must be resolved. In recent years, nano bioceramics have garnered considerable interest in tissue engineering due to their high surface bioactivity.[53] It has also been demonstrated using inductively coupled plasma enhanced chemical vapor deposition (ICPECVD),[54] femtosecond laser,[55] suspension coating and melt bonding techniques[31,56] that the nano-structured silicon nitride coating formed on polyether ether ketone (PEEK) has excellent osteogenic properties. Therefore, when the porous structure cannot be formed as a result of the pursuit of mechanical properties, the construction of nanostructures on the dense silicon nitride surface can increase osteogenic activity. Meanwhile, nanostructures improve the surface roughness and hydrophilicity of silicon nitride, which facilitates the adsorption of free proteins as well as cell adhesion, prolifer-

ation, and differentiation.[57] Moreover, the construction of nanostructures on the surface of bioceramics can reduce the grain size on the surface of the materials, thereby increasing their bending resistance.[32] Therefore, the silicon nitride molding process should not focus solely on the reduction of material porosity and the improvement of material strength; accordingly, the construction of surface micro and nanostructures must also be the subject of future research.

Nano silicon nitride has a number of potential applications, including the construction of nanostructures on the surface of materials and nanoparticles. Similar to other ceramics, nano silicon nitride particles have a large specific surface area, allowing them to release surface-active substances to a greater extent. In addition, nano silicon nitride particles have the ability to transport highly concentrated functional factors that promote bone development and angiogenesis.[58] After entering the damaged area, nano silicon nitride particles are engulfed by damaged repair cells, inducing the differentiation of mesenchymal stem cells and accelerating the formation of an osteogenic microenvironment. Combining nano silicon nitride particles with degradable organic materials (such as GelMA hydrogel) can lead to the development of novel bone tissue engineering scaffolds.[59] After implantation, nano silicon nitride particles and other regenerative factors are secreted to enhance osteogenic properties and degrade gradually. However, there is little research and application of nano silicon nitride particles that can be independently developed and utilized. Compared to other bioceramic nanomaterials, such as nano-hydroxyapatite and bioglass, the influencing factors, such as the effects of particle size on tissue regeneration, degradation performance, and cytotoxicity, which govern the biological effects of nano silicon nitride particles are still unclear.

2.2 Application in clinical orthopedics and dentistry

2.2.1 Spinal spacer. On account of its superior mechanical and biological properties, silicon nitride is predominantly used in orthopedics. Silicon nitride-based implants (including massive dense implants and relatively loose bone engineering scaffolds) and silicon nitride coatings constitute the majority of orthopedic implants. Silicon nitride has been successfully utilized as a spinal spacer in the treatment of lumbar disc inflammation during spinal reconstruction.[16,60–62] In addition, the fusion rate of porous silicon nitride and PEEK at 24 months for anterior cervical discectomy and fusion was compared in a single-blind randomized controlled trial.[63] At 24 months post-surgery, both groups had achieved complete fusion. Currently, autograft-filled porous PEEK scaffold spacers are the most commonly used material for spinal fusion in the clinic. The comparable fusion rates of the two spacers indicate that silicon nitride can be used as a new material for spinal spacers. However, silicon nitride is superior to PEEK for inducing osteogenesis. Due to the bone-inducing properties of silicon nitride, autograft is not required for bone regeneration during surgery. Concurrently, silicon nitride possesses partial radiation resistance, which can develop in radiography but does not result in artifacts.[64]

2.2.2 Joint replacement. Although the safety and efficacy of silicon nitride joint prostheses have been confirmed *in vivo*,[65,66] there have been no clinically significant cases reported. The dense, massive silicon nitride joint replacement can create a smooth and hard contact surface for the joint, as well as a porous structure for the embedded bone portion. The excellent osteogenic induction ability of silicon nitride can promote the rapid growth of bone tissue into a porous structure.[60] Therefore, silicon nitride joint prostheses can achieve adequate early stability and enhance the surgical success rate. However, although the hardness of silicon nitride can reach 13–16 GPa, it is still less than the hardness of other ceramic joint prostheses (alumina base, zirconia base, *etc.*).[35] Silicon nitride has excellent friction resistance and one of the lowest wear rates among orthopedic joint replacement materials currently available, which is precisely what joint prosthesis materials require.[67] In addition, silicon nitride wear particles have a low immune response and can be slowly dissolved in polar liquids like PBS and bovine serum, allowing them to be absorbed *in vivo*, thereby reducing the risk of aseptic loosening.[68] Even without preparing dense silicon nitride implants, the excellent osteogenic properties of silicon nitride can be fully utilized by applying a silicon nitride coating to the surface of existing joint prosthesis materials.

2.2.3 Fixation nail and plate. In oral and maxillofacial surgery, including jaw fracture fixation and fibula flap transplantation, retentive nails and plates are utilized to form solid internal fixation and accelerate fracture healing. Currently, in clinical practice, retainer plates and nails made of silicon nitride not only play a fixed role but also release active substances at the broken end of the fracture, accelerate osteogenic differentiation, and thus accelerate the healing of the broken end of the fracture. In addition, the lack of magnetism and partial radiation resistance in the use of X-rays facilitate imaging of the oral and maxillofacial head-and-neck region.

2.2.4 Dental implant. Exploring novel materials for dental implantology has always been one of the hottest topics in the field of oral implantology. Silicon nitride possesses the requisite pliability, biocompatibility, and wear resistance for dental implant materials. Compared to titanium implants, which are widely used in clinics today, silicon nitride materials have the following advantages: a superior osteogenic performance, an elastic modulus closer to the jaw, antibacterial performance, non-magnetic nature, no metal artifacts during image examination, no foreign body reaction due to metal wear particles, and beautiful appearance.[69] Thus, silicon nitride is expected to be a new dental implant material. Although ceramic materials such as zirconia[70,71] have been used in dental implant construction for a long time, their brittleness and insufficient strength make them difficult to popularize; this is also one of the challenges faced by pure silicon nitride dental implants. Additionally, dental implants have stringent requirements for precision and strength, necessitating the urgent solution of the problem of rapidly shaping silicon nitride

implants with high toughness, hardness, and precision. In addition, although DLP has been used to manufacture silicon nitride, and its *in vitro* mechanical properties and biocompatibility have been evaluated, its applicability to personalized implant design in clinical settings requires additional comprehensive evaluation of its *in vivo* safety and efficacy.[72]

3. Effects of silicon nitride on the biology of the osteogenic microenvironment and osteogenic mechanisms

The rate of bone formation is affected by the local osteogenic microenvironment. The interaction between osteogenic factors and osteogenesis-related cells in the osteogenic microenvironment jointly promoted the osteogenic differentiation of mesenchymal stem cells.[73] In addition, local vascular regeneration and immune inflammation are also involved in the repair of local bone tissue. Silicon nitride can effectively contribute to bone regeneration and repair by synthesizing and releasing a series of substances, such as $Si(OH)_4$, NO and other silicon-containing ionic groups, through its specific surface chemistry in solution, thereby regulating the above-mentioned biological functions, as described in detail below[9,27,74–76] (related research see in Table 1 and overview on mechanism of silicon nitride osteogenic properties see in Fig. 1).

3.1 Effects on bone formation

3.1.1 Activation of redox signaling. Si-OH is one of the major functional groups on the surface of silicon nitride materials. $Si–O^-$ superoxide ions are formed and free electrons are released through acid–base equilibrium reactions.[77] Superoxide ions oxidize ammonia to hydroxylamine (NH_2OH), while the oxygen in hydroxylamine further absorbs free electrons and combines with free protons to reduce to H_2O. Moreover, N is further oxidized to NO_2^-, while HNO_2 and NH_2OH can be further reacted to produce NO, which can also be oxidized to –OO–N═O by the O_2^- generated from silicon nitride. NO_2^-, NO, and others are all RNS.[27] Under oxidative stress, RNS effectively regulate the relevant signaling pathways of osteoblasts, BMSCs, and other cells, and exert positive effects on bone formation. NO, as the main component of RNS, has a dual regulatory effect on osteoblast-related cells, *i.e.*, low concentrations of NO can promote the proliferation and differentiation of osteoblasts, while high concentrations of NO manifest an inhibitory effect on osteoblasts.[78] However, silicon nitride can be a stable NO donor in aqueous solution and that the amount of NO released from its surface is in the safe zone of being able to promote osteoblast proliferation, thus reducing the risk of high concentrations of NO inhibiting bone formation.[9,77]

It has been proved that upon mechanical stimulation, osteoblasts can produce endogenous NOS2 and are expected to promote their own proliferation and differentiation through the NOS2/NO/COX2 pathway,[27,79,80] while COX2 can also promote PGE2 production, which acts on the PGE2 bone receptors (EP2 and EP4) of osteoblast precursors to promote

Table 1 Effect matters released by Si_3N_4 and their effects on osteogenesis, experience models and pathway or targets

Effect matter	Effect on osteogenesis promotion	Experiment model	Pathway/target	Ref.
NO	Promote BMSCs osteogenic differentiation	KUSA-A1 mesenchymal cells	NOS2/NO/COX2	27
NO	Promote BMSCs osteogenic differentiation	OVX mice	NO/cGMP/PKG Wnt/β-catenin	82
–OO–N═O	Osteoclast differentiation up-regulated by redox signal	BMMs	8-Nitro-cGMP/RANKL	84
H_4SiO_4	Induce osteoblast autophagy	Osteoblast-like cell lines MG-63 and U2-OS	PI3K/AKT/mTOR	93
Si^{4+}	BMSCs autophagy	BMSCs	AMPK/mTOR/ULK	90
H_4SiO_4	Induce osteoblast autophagy	Murine preosteoblast MC3T3-E1	—	89
H_4SiO_4	Induce osteoblast differentiation	Murine preosteoblast MC3T3-E1	Wnt/β-catenin	92
OPG	Induce preosteoclast differentiation	BMMs	AMPK/mTOR/p70S6K	88
NO	Promote BMSCs metabolism and osteogenic differentiation	KUSA-A1 mesenchymal cells	—	9
Si^{4+}	Promote M2 phenotype polarization of macrophages	BMDMs	—	106
Si^{4+}	Promoting M2 phenotype polarization of macrophages and immunosuppression	HBMSCs	—	107
Si^{4+}	Promoting the apoptosis of macrophages	RAW264.7 cells	MAPK and NF-κB	108
H_4SiO_4	Inhibiting osteoclast differentiation	RAW264.7 cells	RANK/RANKL	109
Si_3N_4	Inhibiting osteoclast differentiation	SaOS-2 cells	RANK/RANKL	110
NO	Promote M2 phenotype polarization of macrophages	eNOS transgenic mice	NO/VASP	102
Si_3N_4	Promoting angiogenesis and osteogenesis	Murine preosteoblast MC3T3-E1	—	31
Si^{4+}	Promoting angiogenesis	HDF/HUVEC, BMSC/HUVEC co-culture system	VEGF/KDR/eNOS/NO	118 and 119
Si^{4+}	Promoting HUVECs migration	HUVECs	—	117
NO	Dilating blood vessels	HUVECs	cGMP/cGKI	117

their further differentiation.[27,81] Thus, the osteogenic effect is inevitably promoted by the regulatory role of the NO released from the surface of silicon nitride. Moreover, the effect of NO on osteoblasts is primarily regulated through upregulation of cyclic guanosine monophosphate (cGMP) and the downstream signaling molecule protein kinase G (PKG), *i.e.*, the NO/cGMP/PKG pathway. Either the eNOS gene or PKG gene in rats exhibit abnormal bone development after knockdown, indicating the importance of this pathway in bone formation and the low concentration of NO from eNOS is involved in the regulation of this pathway. Silicon nitride also released low concentration of NO, which may directly up-regulate the activity of downstream factor cGMP/PKG.[78] PKG1 and PKG2 are the primary downstream effector proteins of cGMP. PKG2 promotes osteoblast proliferation by activating Src, Erk1/2, Akt, and other signaling molecules, and inhibits apoptosis in concert with the PKG1 isoform PKG1α. Meanwhile, the upregulated Akt kinase also activates the Wnt/β-catenin pathway to promote osteoblast differentiation.[82] On the other hand, regarding the effects of NO on bone formation, it inhibits osteoclasts, in addition to its positive effects on osteoblasts. Nuclear factor-κB receptor activator ligand (RANKL) is a key stimulator of osteoclasts.[83] Studies have revealed that after exogenous supplementation of NO, RANKL expression decreased, while its antagonist osteoprotegerin (OPG) expression increased. The decrease in the RANKL/OPG ratio led to the inhibition of nuclear factor-κB (NF-κB) activation, which subsequently inhibited osteoclast recruitment and differentiation. Unlike the regulation of NO in osteoblasts, it has been verified that the cGMP/PKG pathway is not involved in the regulation of NO, which affects the RANKL/OPG ratio, and the exact molecular mechanism remains to be discovered and verified.[84,85]

However, another study found that –OO–N═O, the reaction product of NO, promoted RANKL-induced osteoclast differentiation by nitrating GTP to 8-nitro-GTP, which was subsequently metabolized by soluble guanylate cyclases (sGC) to 8-nitro-cGMP complexes.[86] However, in this study, we directly applied 8-nitro-cGMP to stimulate the cells, and the dose and concentration of NO feedstock could not be clarified. Thus, the effects of NO on bone formation could not be directly investigated. Nonetheless, it also suggested that other active factors (*e.g.*, –OO–N═O) produced on the surface of silicon nitride, in addition to NO, may exhibit different effects on osteogenesis. Determining how other ions or groups (such as –OO–N═O, NO_2^-) released from silicon nitride regulate the cellular behavior of osteogenesis-related cells is also a key direction for future research (see in Fig. 2).

3.1.2 Induction of autophagy in osteogenesis-related cells. Autophagy is an intracellular metabolic behavior in which cells encapsulate proteins or organelles in the cytoplasm through vesicles and then bind to lysosomes, forming autophagic lysosomes that degrade their encapsulated contents, thereby achieving the cell's own metabolic needs and the renewal of certain organelles.[87] There is a close relationship between cellular autophagy and osteogenesis, which is

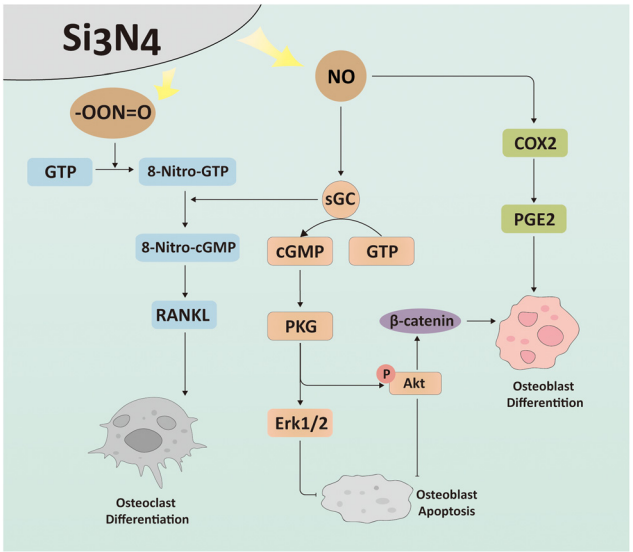

Fig. 2 Si_3N_4 activates redox signaling to promote osteogenesis. NO derived from silicon nitride surface promotes osteoblast differentiation by enhancing COX2/PGE2 signaling, and promotes osteoblast proliferation and inhibited apoptosis by activating cGMP/PKG signaling.

reflected in the promotion of bone formation and inhibition of bone resorption induced by enhanced autophagy in osteogenesis-related cells.[88] The expression levels of osteogenic markers such as RUNX2 and COL1 were significantly increased in osteoblasts under treatment with the autophagy inducer rapamycin, and the ALP activity was also enhanced, indicating that enhanced osteoblast autophagy promoted osteogenic differentiation.[89] Moreover, the promotion of osteogenic differentiation further induced osteoclast autophagy and inhibited osteoclast differentiation.[90] In other words, the induction of osteoblast-associated cell autophagy promoted osteogenesis.

The current study initially found that orthosilicic acid generated from the hydrolysis of the silicon nitride surface induced autophagy in osteoblasts, bone marrow mesenchymal stem cells (BMSCs), and osteoclasts, among others. Orthosilicic acid stimulated preosteoblasts led to enhanced autophagy, along with the promotion of osteogenic differentiation and mineral formation. However, the molecular mechanism of orthosilicic acid promoting autophagy of osteoblasts remains unclear.[91] After treated by calcium silicate material (the main active component includes SiO_4^{4-}), the AMPK/mTOR/ULK1 pathway in BMSCs was activated to induce autophagy, and the end result was also an enhancement of osteogenic differentiation.[92] Meanwhile, the activation of AMPK signaling in osteoblasts attenuates apoptosis.[93] In addition, orthosilicic acid upregulates the Wnt activity and increases the OPG expression through the Wnt/β-catenin pathway,[94] while OPG promotes osteoclast precursor autophagy through the AMPK/mTOR/p70S6K signaling pathway, which plays a key role in the inhibition of osteoclast differentiation and bone resorption.[90] The previous study suggested that NO can also upregulate OPG expression, and it is not difficult to find that the two

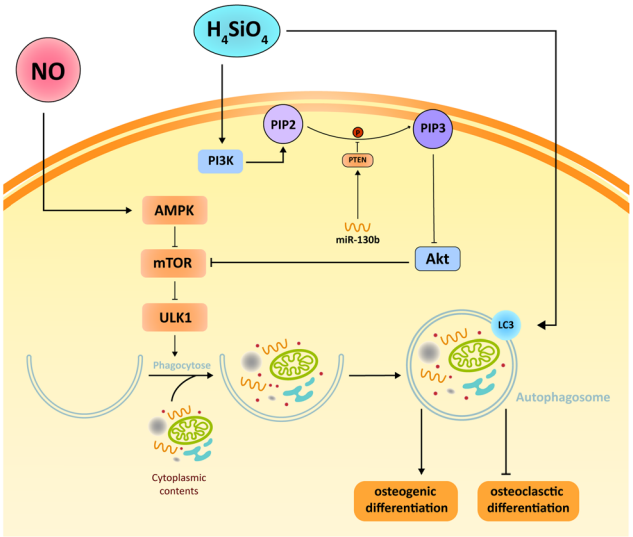

Fig. 3 H_4SiO_4 released by Si_3N_4 regulates PI3K/Akt signaling, and NO regulates AMPK signaling, which jointly inhibit mTOR activity, resulting in enhanced autophagy. The expression of LC3 is also directly enhanced by H_4SiO_4.

hydrolysis products of silicon nitride can play synergistic roles in regulating osteoclast autophagy (see in Fig. 3).

Taken together, it can be found that both orthosilicic acid and NO released from the surface of silicon nitride induce autophagy in osteoblasts and osteoclasts, promote osteogenic differentiation, and inhibit osteoclastic differentiation. Ultimately, osteogenesis is accelerated. However, there is insufficient direct evidence for the association of silicon nitride with the induction of osteogenesis-related cell autophagy to promote osteogenesis. Regarding silicon nitride and the induction of autophagy to promote osteogenesis, the following questions remain to be addressed. Although there are numerous signaling pathways involved in the key factor for autophagy (mTOR/ULK), does orthosilicic acid induce autophagy through these pathways or are there other potential targets or pathways? Meanwhile, studies have demonstrated that orthosilicic acid increases the expression of miR-130b, which directly targets PTEN, and that the inhibition of PTEN can significantly up-regulate the downstream factors PI3K and Akt, leading to an increase in mTOR activity.[89,95,96] Enhanced mTOR activity inhibits autophagy, despite the fact that the final product also promotes osteogenic differentiation. However, the regulation of autophagy of osteoblast-related cells by orthosilicic acid released from the surface of silicon nitride may be bidirectional, involving variables such as the concentration of orthosilicic acid. Moreover, it is also possible that the autophagy may be induced by oxidative stress after silicon nitride particles are absorbed by cells. The autophagy mechanism is complicated and involves numerous factors. In addition to the key factor (mTOR/ULK) mentioned above, so other key factors such as Atg and the BPI3KC3/Beclin-1 complex mediate the signaling pathways associated with silicon nitride-induced autophagy? Whether other surface factors of silicon nitride, such as NH_3 and NH_2OH, are also involved in regulating autophagy-related pathways remains to be investigated. Autophagy can be broadly classified into macroautophagy, microautophagy, and molecular chaperone-mediated autophagy.[87] Most studies on silicon nitride-induced autophagy involve macroautophagy, while the latter two types of autophagy are less studied. Selective autophagy such as mitophagy has also been found to play a key regulatory role in bone metabolic diseases. It is yet to be determined whether silicon nitride also induces mitochondrial autophagy.[97] These are questions that need to be addressed in the future.

3.1.3 Enhancement of glycolysis in osteogenesis-related cells. Despite the presence of sufficient oxygen, aerobic glycolysis is the primary energy source for osteoblast differentiation in mature osteoblasts with mature mitochondria.[98] In addition, lactate, which is a byproduct of aerobic glycolysis, increases IGF-1 and osteocalcin expression in the osteoblast cell lines.[99] Thus, the enhanced aerobic glycolysis of osteoblasts helps promote osteogenic differentiation.

NO released on the surface of silicon nitride may regulate the aerobic glycolysis ability of pluripotent stem cells capable of osteogenic differentiation, thereby enhancing osteogenic differentiation capacity.[100] A study revealed the enhanced effects of silicon nitride on the metabolism of BMSCs: high ATP levels were observed in BMSCs co-cultured with silicon nitride. With a linear decrease in the ATP/ADP ratio with the culture time, Raman spectroscopy demonstrated a significant decrease in tryptophan, along with detectable increases in IGF-1 and osteocalcin.[9] The insufficient production of NO also down-regulated the expression of PFKFB3, PKM, and other glycolytic-related genes in bone marrow stromal cells, resulting in diminished glycolytic activity. However, after supplementation with NO, glycolysis ability was fully restored.[100] Enhancing aerobic glycolysis in osteogenesis-related cells may be another important approach by which silicon nitride promotes osteogenesis. However, this is merely a hypothesis based on a small amount of available evidence. The following points remain to be further verified: (i) whether silicon nitride enhances the aerobic glycolysis of osteoblast cell lines through increased NO release and whether any additional substance released from the surface of silicon nitride is involved; and (ii) the associated molecular mechanisms for the enhanced aerobic glycolysis of osteoblast cell lines and enhanced osteogenic differentiation. These may represent directions for future research.

3.1.4 Accelerated osteoblast-mediated bone mineralization. Osteoblast-mediated bone mineralization is initiated by an extracellular vesicle matrix vesicle that contains transporters and enzymes. These transporters and enzymes work together to allow ions such as Ca^{2+} or PO_4^{3-} to enter the vesicle and gradually form hydroxyapatite (HAp) crystals, which, together with subsequent collagen mineralization, is referred to as primary bone mineralization.[101] Studies have revealed that the SiO_4^{4-} and N released from silicon nitride are integrated into apatite by osteoblasts on the surface of the silicon nitride material, replacing some of the PO_4^{3-} and OH^- (or O), respectively.[76] This may occur as a result of higher concentrations of

SiO_4^{4-} and N being released from the silicon nitride, transported into matrix vesicles, and thus integrated into apatite crystals. This transition-state hydroxyapatite forms faster than normal apatite, which directly increases the rate of bone mineralization. In addition, the presence of SiO_4^{4-} tetrahedra and N allows bone apatite to provide a favorable chemical interface for osteoblast activity, including various surface charges, which enhances surface protein folding, cell motility, and proliferation, along with osteogenesis-related cells signaling on the material.[102,103]

3.2 Effects on immunomodulation

The immune system is not only involved in maintaining bone homeostasis, but also plays a crucial role in the repair of bone defects. Immune cells are involved in the entire process of tissue repair: chemokines and cytokines secreted by inflammatory cells attract mesenchymal stem cells to the site of injury and control the osteoclastic and osteogenic processes.[104] Bone regeneration materials should be developed to modulate the host immune response and more effectively promote bone repair when implanted in the body. Macrophages are some of the cells that play key roles in osteogenesis, not only as immune regulators, but also as precursor cells that differentiate into osteoclasts under the action of the RANK/RANKL pathway.[105] Currently, the key to bone immunomodulatory biomaterials lies in the induction of different macrophage activities.[104,106,107] The novel biomaterial silicon nitride may also possess some immunomodulatory ability (see in Fig. 4).

Although there have been no direct studies that demonstrated the ability of silicon nitride to modulate the host immune response, both the Si ions and NO produced by silicon nitride in its interaction with the biological environment are closely related to the regulation of immune inflammation. Silicate materials promote macrophage M2 polarization, inhibit M1 polarization, and release anti-inflammatory factors such as IL-10 and TGF-β to promote the osteogenic differentiation of BMSCs. Moreover, M2 macrophages also act on BMSCs to promote osteogenic differentiation through the release of oncostatin M (OSM).[108] Studies have shown that silicate materials can further enhance the promotion of macrophage M2 phenotype polarization *via* MSCs' medium. Silicate ions released by silicate inhibit inflammatory MAPK and NF-B signaling pathways *via* a caspase-dependent pathway, thereby promoting macrophage apoptosis; the decrease in local macrophages attenuated local inflammation[109,110] In addition, the decrease in macrophages indicated a decrease in osteoclast differentiation, which was consistent with numerous previous findings. Moreover, under the influence of silicon nitride, the concentration of sRANKL decreased, and the RANKL pathway-dependent osteoclast differentiation was significantly inhibited, as demonstrated by these findings.[111,112] Therefore, silicon can directly or indirectly mediate the interaction between macrophages and BMSCs and modulate the effects of macrophages on osteoimmunity. Additionally, it inhibits osteoclastic differentiation and reduces inflammation, thereby promoting osteogenesis directly or indirectly. Moreover, eNOS-derived NO has long been recognized as one of the key factors involved in the regulation of organismal immunity.[113] In early osteogenesis, the NO produced by silicon nitride can control local inflammation by removing the microorganisms of damaged DNA, as well as disrupted cell membranes.[9,77,114] A host's BMSC-mediated immunity can also be suppressed by NO.[115] eNOS-derived NO promoted the M2 phenotypic polarization of macrophages *via* the downstream signaling factor VASP. Silicon nitride, like silicate biomaterials, produces silicon-containing ionic groups. Thus, it can be speculated that silicon nitride also has the immunomodulatory function of silicate materials. In addition, because of the involvement of exogenous NO, silicon nitride may have a stronger immunomodulatory ability than conventional biomaterials such as CaP and CS materials.

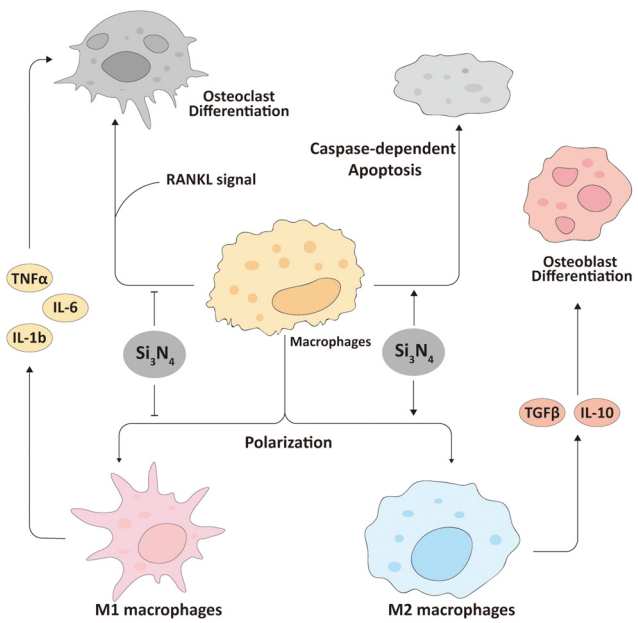

Fig. 4 Si_3N_4 is involved in immune regulation of bone regeneration microenvironment.

At present, studies on the immunomodulatory ability of silicon nitride mainly focuses on its effect of surface bioactive ions on macrophages. And there is a lack of relevant studies on other immunomodulatory cells such as lymphocytes and dendritic cells. The RANKL pathway regulated by silicon nitride plays an important regulatory role in osteoimmune, and RANKL/RANK has been shown to be associated with dendritic cell survival[116] and lymphocyte development.[117] Silicon nitride is likely to have the ability to regulate these cells, which will be the future direction of further research in this field and provide new insights into immunomodulation of silicon nitride.

3.3 Effects on vascular regeneration

The reconstruction of blood flow patterns is essential for tissue repair and bone regeneration. The complex crosstalk mechanism between angiogenesis and osteogenesis constitu-

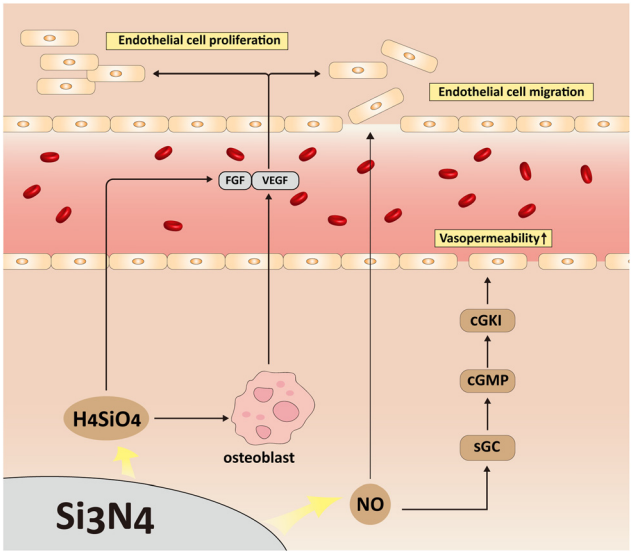

Fig. 5 Si_3N_4 promotes angiogenesis by increasing vascular permeability and inducing endothelial cell proliferation and migration.

tes an "angiogenic–osteogenic coupling", which is essential for tissue repair and bone regeneration[118] (see in Fig. 5). Dai[31] prepared silicon nitride coated PPK (CSNPPK) by suspension coating and melt binding, which showed more bone regeneration and angiogenesis than the control group (PPK) after implantation into rabbit femurs. In addition, significantly higher levels of VEGF expression were detected in the experimental group than in the control group. Therefore, it is certain that silicon nitride possesses promoting effects on vascular regeneration. This verified that silicon nitride can promote vascular regeneration.

VEGF, which is a key factor in angiogenesis–osteogenesis coupling,[119] is regulated by Si ions released upon the dissociation of silicon nitride in an aqueous solution. This in turn regulates the osteogenic microenvironment, which involves multiple cells *in vivo*. It was shown that Si ion-mediated intercellular paracrine synergy promoted pro-angiogenesis. In a co-culture system of human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs), as well as in a co-culture system of BMSCs and HUVECs, Si ions greatly promoted the ability of HDF and BMSC to secrete VEGF, and then upregulated the expression of the VEGF receptor KDR in HUVECs through paracrine effects, thereby activating the VEGF/KDR/eNOS/NO axis to promote angiogenesis.[120,121] Moreover, Si ions can also act directly on HUVECs. On one hand, they upregulate the expression of hypoxia inducible factor 1α (HIF 1α), which in turn promotes the expression of bFGF, VEGF, and eNOS, thereby enhancing the migration and tube-forming properties of HUVECs.[119] On the other hand, they promote the angiogenic effect by upregulating the PI3k/Akt pathway, which in turn promotes the angiogenic effects.[122] Based on previous studies, we can speculate that silicon nitride can regulate both osteogenesis and angiogenesis, two highly coupled physiological processes, through its product, orthosilicicic acid, *in vivo via* the PI3k/Akt/HIF-1α axis. However, this remains to be proven.

NO plays a key role in the process of angiogenesis. In addition to the aforementioned NO production induced by the Si ion/VEGF axis, silicon nitride can also release NO directly in solution, which may have a pro-angiogenic effect. It can regulate the apoptosis and migration of endothelial cells, and promote local blood flow through vasodilation effect of high cGMP/cGKI level, which are conducive to angiogenesis.[123–125] However, there have been insufficient studies to directly prove the effects of silicon nitride on vascular endothelial cell activity. Moreover, the specific molecular mechanisms by which Si regulates the expression of angiogenic-related factors are unknown.

4. Main factors affecting the performance of silicon nitride osteogenesis

4.1 Wettability

Hydrophilicity and hydrophobicity are expressions of a material's wettability properties. Its wettability has a significant effect on the biological properties of an implantable material, *e.g.*, hydrophilic surfaces facilitate protein adsorption.[126,127] Hydrophilic implant surfaces are more easily wettable by body fluids, and free proteins from body fluids are thus adsorbed to the implant surface along with body fluids. The initial interaction of proteins and the implant surface may affect the adhesion, proliferation, migration, and differentiation of the initial human osteoblasts.[128] The surfaces of silicon nitride materials contain large amounts of Si-NH_2 and Si-OH. The hydroxyl and amino groups are polar groups, which make silicon nitride highly hydrophilic.[12] The molecular model of the BMP-2 matrix adsorption behavior shows that hydrophilic silicon nitride has a strong adsorption effect on BMP-2, which indicates that the hydrophilicity of silicon nitride contributes to its osteogenic properties.[129] Moreover, the enhanced hydrophilicity of the silicon nitride surface improves the adhesion of human cells.[130] After implantation, due to its strong hydrophilic surface, silicon nitride can be quickly moistened by body fluids which are rich in a large number of proteins and related cells that can trigger bone repair, so the early osteointegration rate will be greatly increased.

Two strategies can be adopted to regulate the hydrophilicity of silicon nitride, one is increasing the hydrophilicity by increasing the surface roughness, and another is increasing the surface hydrophilic groups such as the carboxyl, hydroxyl, and amino groups.[130–133] Nitrogen heat treatment increases the bonding of the nitrogen to the surface of the silicon nitride, as well as the number of surface amino groups. Air heat treatment thickens the oxide layer. Yet, the surface oxidation increases the hydroxyl groups. When HF acid is used for nanoscale etching, the surface oxide layer is dissolved,

which exposes the inherent amino groups of the silicon nitride.[134]

4.2 Surface roughness

The surface roughness of an implant material affects bone growth on the implant surface.[135] Thus, modulating the surface roughness to promote bone regeneration has now been applied to some implants such as titanium implants.[136] A large surface roughness means a larger surface area and more space for cell attachment, which can lead to greater cell adhesion, thereby indirectly affecting the cell proliferation capacity. After implantation, osteoblasts rapidly interact with the implant surface matrix *via* integrins, which cause cells to form focal adhesions. More focal adhesions change the morphology of the cells.[137] The morphological diversification actually promotes the ability of the cells to differentiate.[138] Studies have also shown that silicon nitride implant surfaces with a high surface roughness exhibit higher osteogenic efficiency.[134,139,140] The surface texture and roughness also affect the surface wettability, which is enhanced by increasing the surface roughness, *i.e.*, hydrophilic surfaces are more hydrophilic, while hydrophobic surfaces are more hydrophobic.[134,141] Changing the surface roughness of silicon nitride *via* abrasive blasting, chemical etching, *etc.*, is an effective method for adjusting the material's capacity to induce osteogenesis. In addition, because the surface of silicon nitride exhibits hydrophilicity, increasing its surface roughness may further increase the surface hydrophilicity, which has a positive effect on osteogenesis.

4.3 Surface charge

The pH of a material with a net charge of zero on its surface is the isoelectric point (IEP) of that material, which is 9.3–9.7 for pure silicon nitride. Hence, in theory, the surface of a pure silicon nitride material is generally positively charged. However, silicon nitride is zwitterionic, which may be because the surface of silicon nitride is rich in positively charged amine groups and negatively charged deprotonated silanol,[142] or possibly Y-OH and Al-OH from sintering additives (Al_2O_3 and Y_2O_3).[143] However, if silicon nitride is exposed to an aerobic environment, Si–O–N and Si–O groups will be generated in turn, and an oxide layer (primarily composed of SiO_2) will gradually form on the surface. Therefore, the surface of silicon nitride in the humoral environment is rich in silanol groups. The surface charge of silicon nitride is mainly affected by the silyl alcohol group, and a small amount of amine group will also play a role in changing the surrounding pH value, and these two groups can affect the surface charge of silicon nitride mutually. The isoelectric point of pure SiO_2 is 2–3, which implies that the surface is negatively charged at a physiological pH. If the O/Si on a silicon nitride surface is increased to greater than the N/Si, the surface of the implanted material will have a negative charge.[144,145] Around materials similar to silicon nitride, apatite spontaneously deposits on the surface of a material with a negative surface charge, which facilitates osseointegration.[134]

However, it has been revealed that an implant material with a positive surface charge facilitates cell apposition and promotes the adherence of various proteins, owing to the fact that cell surfaces and proteins are generally negatively charged, and this non-specific attraction promotes osteogenesis.[126] This phenomenon of promoting osteogenesis may also be the result of the surface charge regulating the cell's signaling pathways. The expression of NOS on the surface of BMSCs is affected by the local electrical environment. When BMSCs are in a positive electrical environment, their NOSs are mostly expressed as iNOS, and further NO production is involved in the promotion of osteogenic differentiation.[146,147] However, if the surface charge is excessively high, excessive immunoregulation is generated, which counteracts the aforementioned responses.[148]

It is certain that changes in the charge carried by the surface of silicon nitride affect its osteogenic properties. There is no doubt that when the surface charge of the material is positive, osteogenic differentiation is enhanced owing to the regulation of various cell and protein adhesion and signaling pathways. In contrast, when it exhibits a negative charge, osteogenesis is also promoted, but the mechanism may be the accelerated deposition of apatite. Further research is required to determine the difference in the effects of positive and negative surface charges. The persistent swing in the formation of positively and negatively charged sites on the surface of silicon nitride have been shown to be the impetus for the deposition of hydroxyapatite at its surface. N-vacancies and N–N bonds are formed on the surface of silicon nitride by nitrogen annealing test, so that the surface stoichiometry of silicon nitride is modified appropriately and the cell metabolism is enhanced. Therefore, the formation of surface charged sites induced by various means can effectively change the surface osteoinductivity of silicon nitride. To sum up, it is extremely important to investigate the surface charge points that maximize the osteogenic effect of silicon nitride materials.

5. Shortcomings and outlook

Overall, silicon nitride has good bone regeneration-promoting properties owing to its unique surface chemistry, which, combined with its unique antibacterial properties, make it an ideal replacement material for human hard tissues.

The research on the mechanisms related to the performance of silicon nitride remains at a preliminary stage. Osteogenesis is a complex process. In addition to the direct participation of skeletal functional cells, the formation of blood vessels and inflammatory responses also play important indirect roles. Most of the recent studies on the osteogenic properties of silicon nitride have addressed the phenomena related to cellular activities, with less in-depth study devoted to the molecular mechanisms. As a bioceramic material that effectively promotes osteogenesis, there is still much room for research on the mechanism and applications of silicon nitride.

The primary research shortcomings at present include the following.

(I) The construction of micro–nano structures on the surface of silicon nitride has been reported to successfully improve the bone regeneration performance of silicon nitride, indicating that nano silicon nitride is likely to yield exceptionally brilliant results in biomedicine. How to better utilize nano silicon nitride merits further discussion, as it will be crucial for the future development of new bone defect repair materials. It has been suggested that nanoparticles, when ingested by BMSCs, form protein crowns that are mistakenly perceived by cells as misfolded proteins and activate autophagy, thereby enhancing osteogenic differentiation. Silicon nitride nanoparticles may have a similar effect. Still, the potential cytotoxicity to cells when silicon nitride nanoparticles are not cleared in time after entering the body cannot be ignored in this regard. The cytotoxicity of silicon nitride may be caused by the direct uptake of silicon nitride nanoparticles by cells *via* cytophagy. Subsequently, the release of RNS by dissociation in the cytoplasm may directly cause intracellular lipid oxidation, DNA damage, and protein degeneration, as well as a reduction in the mitochondrial membrane potential, resulting in mitochondrial membrane damage and, ultimately cell death. However, the precise effects of the cytotoxicity of nanoparticles of silicon nitride require additional research. Moreover, the development of nano silicon nitride bone defect repair materials require a large number of subsequent *in vivo* and *in vitro* experiments, including the determination of preparation methods, mechanical properties, the exploration of the optimal pore structure and porosity, and the effect of silicon nitride concentration in products.

(II) Despite the fact that silicon nitride surface substances have been linked to numerous osteogenesis-related molecular mechanisms, there have been insufficient *in vivo* and *in vitro* studies demonstrating the effects of silicon nitride on the formation of an osteogenic microenvironment. Moreover, gaps also exist in the in-depth mechanistic studies that link these active substances to related signaling pathway factors. For example, orthosilicic acid can upregulate the Wnt/β-catenin pathway to promote osteogenic differentiation; it can also inhibit osteoclast differentiation by upregulating miR-146a expression, leading to the blockage of the NF-kB activation process.[149] However, the regulatory mechanism between orthosilicic acid and the initial regulators of signaling pathways such as Wnt and miR-146a remains unclear. Furthermore, the bioactive substances on the surface of silicon nitride result from the dissociation of silicon and nitrogen in an aqueous solution. The majority of research on bioactive substances on the surface of silicon nitride has focused on nitrogen-related molecules, with less research conducted on silicon-related molecules and their potential synergistic effects. In addition, the bioactive substances on the surface of silicon nitride result from the dissociation of silicon and nitrogen in an aqueous solution. In this regard, although studies on nitrogen-related substances such as NO, NH_3, and NH_4^+ have been carried out, research on silicon-related substances has been largely limited. Therefore, other components of silicon extract besides orthosilicic acid must be clarified further.

(III) Silicon nitride's role in regulating autophagy, glycolysis, and other cellular activities of osteogenesis-related cells needs further investigation. Furthermore, increased autophagy and glycolysis in these osteogenesis-related cells may affect additional cellular activities.

For example, in osteogenesis, autophagic vesicles can act as matrix vesicles secreted by osteoblasts in bone mineralization, thereby enhancing the rate of bone mineral deposition. The glycolytic metabolite lactate also enhances the rate of bone mineralization by increasing osteocalcin expression. The autophagy and glycolysis of osteogenesis-associated cells may be key osteogenic mechanisms, but a direct link with silicon nitride has not been established. Most of the recent studies have involved the regulation of the autophagy and glycolysis by substances released from the surface of silicon nitride. Based on this foundation, future studies can try to establish a direct link between silicon nitride and these mechanisms. In addition, although silicon nitride can only produce low concentrations of RNS, the effects of exogenous RNS on mitochondria, endoplasmic reticulum, and other organelles in normal cells cannot be ignored.

The mechanism of silicon nitride osteogenesis summarized in this paper is mainly reflected in the regulation of cell activity by the surface bioactive substances, which involves multiple signaling pathways. Although recent studies have been limited in their ability to reveal the osteogenic mechanism of silicon nitride, it is undeniable that silicon nitride has a tremendous amount of potential for future applications. Thus, to gain a deeper understanding of the performance of silicon nitride, systematic and standardized research on biological effect and underlying mechanism is, therefore, necessary. In addition, silicon nitride artificial implant materials will be exposed to biological fluid environment and micromechanical conditions, surface oxidation and corrosion of silicon nitride implants are inevitable due to exposure to body fluid environment. The precise stability of their surface stoichiometric behavior must be considered when designing. Correspondingly, future in-depth studies can provide reliable and robust evidence to remove the obstacles that currently impede the development of its applications and play a crucial role in fostering its potential biomedical applications.

Author contributions

Ziyi Liu: conceptualization, investigation, writing – original draft. Ruijie Wang: conceptualization, investigation, writing – original draft. Wenjing Liu: writing – review & editing. Yushan Liu: investigation, writing – original draft. Fujian Zhao: writing – review & editing. Xiaoli Feng: writing – review & editing. Pei Chen: visualization. Longquan Shao: writing – review & editing, supervision. Mingdeng Rong: conceptualization, project administration, funding acquisition, supervision.

Conflicts of interest

The authors declare no conflicts to interests.

Acknowledgements

This study was supported by International Cooperation Project of Science and Technology Planning Project of Guangdong Province (2022A0505050037), Project of Traditional Chinese Medicine Bureau of Guangdong Province (20222142) and Science Research Cultivation program of Stomatological Hospital, Southern Medical University (PY2020010).

References

1 M. Majidinia, A. Sadeghpour and B. Yousefi, *J. Cell Physiol.*, 2018, **233**, 2937–2948.
2 E. García-Gareta, M. J. Coathup and G. W. Blunn, *Bone*, 2015, **81**, 112–121.
3 N. Eliaz and N. Metoki, *Materials*, 2017, **10**, 334.
4 A. Hoppe, N. S. Güldal and A. R. Boccaccini, *Biomaterials*, 2011, **32**, 2757–2774.
5 M. Kaur and K. Singh, *Mater. Sci. Eng., C*, 2019, **102**, 844–862.
6 M. Kaur and K. Singh, *Mater. Sci. Eng., C*, 2019, **102**, 844–862.
7 S. J. Chockattu, D. B. Suryakant and S. Thakur, *Restor. Dent. Endod.*, 2018, **43**, e39.
8 F. L. Riley, *J. Am. Ceram. Soc.*, 2004, **83**, 245–265.
9 G. Pezzotti, *ACS Appl. Mater. Interfaces*, 2019, **11**, 26619–26636.
10 E. Marin, M. Zanocco, F. Boschetto, T. Yamamoto, N. Kanamura, W. Zhu, B. J. McEntire, B. S. Bal, R. Ashida, O. Mazda and G. Pezzotti, *Appl. Sci.*, 2020, **10**, 9039.
11 X. Hu, S. Mei, F. Wang, S. Tang, D. Xie, C. Ding, W. Du, J. Zhao, L. Yang, Z. Wu and J. Wei, *Bioact. Mater.*, 2021, **6**, 3136–3149.
12 T. J. Webster, A. A. Patel, M. N. Rahaman and B. S. Bal, *Acta Biomater.*, 2012, **8**, 4447–4454.
13 C. R. Arciola, D. Campoccia and L. Montanaro, *Nat. Rev. Microbiol.*, 2018, **16**, 397–409.
14 Z. Liu, X. Liu and S. Ramakrishna, *Biotechnol. J.*, 2021, **16**, e2000116.
15 C. Goswami, I. K. Bhat, A. Patnaik, T. Singh and G. Fekete, *Silicon*, 2020, **12**, 1237–1245.
16 M. P. Arts, J. F. C. Wolfs and T. P. Corbin, *Eur. Spine J.*, 2017, **26**, 2372–2379.
17 W. M. Rambo, *Int. J. Surg. Case Rep.*, 2018, **43**, 61–68.
18 B. V. Zhmud and L. Bergström, *J. Colloid Interface Sci.*, 1999, **218**, 582–584.
19 M. Herrmann, *J. Am. Ceram. Soc.*, 2013, **96**, 3009–3022.
20 K. S. McMillan, A. G. McCluskey, A. Sorensen, M. Boyd and M. Zagnoni, *Analyst*, 2016, **141**, 100–110.
21 M. Herrmann, *J. Am. Ceram. Soc.*, 2013, **96**, 3009–3022.
22 B. V. Zhmud and L. Bergstrom, *J. Colloid Interface Sci.*, 1999, **218**, 582–584.
23 L. Mao, L. Xia, J. Chang, J. Liu, L. Jiang, C. Wu and B. Fang, *Acta Biomater.*, 2017, **61**, 217–232.
24 X. Zhou, F. M. Moussa, S. Mankoci, P. Ustriyana, N. Zhang, S. Abdelmagid, J. Molenda, W. L. Murphy, F. F. Safadi and N. Sahai, *Acta Biomater.*, 2016, **39**, 192–202.
25 C. Magnusson, P. Uribe, R. Jugdaohsingh, J. J. Powell, A. Johansson and M. Ransjö, *J. Biomed. Mater. Res., Part A*, 2021, **109**, 1967–1978.
26 Y. You, W. Ma, F. Wang, G. Jiao, L. Zhang, H. Zhou, W. Wu, H. Wang and Y. Chen, *Life Sci.*, 2021, **264**, 118680.
27 G. Pezzotti, T. Adachi, F. Boschetto, W. Zhu, M. Zanocco, E. Marin, B. S. Bal and B. J. McEntire, *Int. J. Mol. Sci.*, 2019, **20**, 4080.
28 Y. Shen, K. Fang, Y. Xiang, K. Xu, L. Yu, J. Chen, P. Ma, K. Cai, X. Shen and J. Liu, *Front. Bioeng. Biotechnol.*, 2022, **10**, 1023032.
29 H. Wu, L. Yang, J. Qian, D. Wang, Y. Pan, X. Wang, N. Saha, T. Tang, J. Zhao and J. Wei, *ACS Biomater. Sci. Eng.*, 2019, **5**, 1290–1301.
30 F. Boschetto, E. Marin, E. Ohgitani, T. Adachi, M. Zanocco, S. Horiguchi, W. Zhu, B. J. McEntire, O. Mazda, B. S. Bal and G. Pezzotti, *Biomed. Mater.*, 2020, **16**, 015015.
31 Y. Dai, L. Chu, Z. Luo, T. Tang, H. Wu, F. Wang, S. Mei, J. Wei, X. Wang and X. Shang, *ACS Biomater. Sci. Eng.*, 2019, **5**, 6425–6435.
32 P. Feng, R. Zhao, W. Tang, F. Yang, H. Tian, S. Peng, H. Pan and C. Shuai, *Adv. Funct. Mater.*, 2023, **33**, 2214726.
33 X. Zhu and Y. Sakka, *Sci. Technol. Adv. Mater.*, 2008, **9**, 033001.
34 M. Rahaman and W. Xiao, *Int. J. Appl. Ceram. Technol.*, 2018, **15**, 861–872.
35 B. S. Bal and M. N. Rahaman, *Acta Biomater.*, 2012, **8**, 2889–2898.
36 F. Zhang, Z. Li, M. Xu, S. Wang, N. Li and J. Yang, *J. Eur. Ceram. Soc.*, 2022, **42**, 3351–3373.
37 J. Rolecek, L. Pejchalova, F. J. Martinez-Vazquez, P. M. Gonzalez and D. Salamon, *J. Eur. Ceram. Soc.*, 2019, **39**, 1595–1602.
38 G. El Chawich, J. El Hayek, V. Rouessac, D. Cot, B. Rebiere, R. Habchi, H. Garay, M. Bechelany, M. Zakhour, P. Miele and C. Salameh, *Materials*, 2022, **15**, 471.
39 J. Cai, B. Zhang, M. Zhang, Y. Wen and X. Qu, *J. Cent. South Univ.*, 2021, **28**, 983–1002.
40 X. Wang, Y. Li, B. Liu, S. Li, W. Duan, G. Wang and F. Chen, *Int. J. Appl. Ceram. Technol.*, 2023, **20**, 1017–1027.
41 P. Yang, Z. Sun, S. Huang, J. Ou, S. Wu, Q. Jiang and D. Li, *J. Asian Ceram. Soc.*, 2022, **10**, 69–82.
42 C. Jia-wei, Z. Bai-cheng, Z. Mao-hang, W. Yao-jie and Q. Xuan-hui, *J. Cent. South Univ.*, 2021, **28**, 983–1002.
43 X. Wang, Y. Li, B. Liu, S. Li, W. Duan, G. Wang and F. Chen, *Int. J. Appl. Ceram. Technol.*, 2023, **20**, 1017–1027.

44 M. A. Sainz, S. Serena, M. Belmonte, P. Miranzo and M. I. Osendi, *Mater. Sci. Eng., C*, 2020, **115**, 110734.
45 Z. Chen, Z. Li, J. Li, C. Liu, C. Lao, Y. Fu, C. Liu, Y. Li, P. Wang and Y. He, *J. Eur. Ceram. Soc.*, 2019, **39**, 661–687.
46 B. Dermeik and N. Travitzky, *Adv. Eng. Mater.*, 2020, **22**, 2000256.
47 S. A. Lurie, Y. O. Solyaev, L. N. Rabinskiy, P. O. Polyakov and I. Sevostianov, *J. Mater. Sci.*, 2018, **53**, 4796–4805.
48 R.-J. Huang, Q.-G. Jiang, H.-D. Wu, Y.-H. Li, W.-Y. Liu, X.-X. Lu and S.-H. Wu, *Ceram. Int.*, 2019, **45**, 5158–5162.
49 X. Du, G. Blugan, T. Kuenniger, S. S. Lee, L. Vladislavova and S. J. Ferguson, *Ceram. Int.*, 2021, **47**, 33525–33536.
50 K. Zhang, Y. Fan, N. Dunne and X. Li, *Regener. Biomater.*, 2018, **5**, 115–124.
51 V. Karageorgiou and D. Kaplan, *Biomaterials*, 2005, **26**, 5474–5491.
52 G. L. Koons, M. Diba and A. G. Mikos, *Nat. Rev. Mater.*, 2020, **5**, 584–603.
53 Y. Cui, H. Li, Y. Li and L. Mao, *Nanoscale Adv.*, 2022, **4**, 334–352.
54 Z. Xu, H. Wu, F. Wang, R. Kaewmanee, Y. Pan, D. Wang, P. Qu, Z. Wang, G. Hu, J. Zhao, R. Zhao and J. Wei, *J. Mater. Chem. B*, 2019, **7**, 6035–6047.
55 H. Wu, T. Liu, Z. Xu, J. Qian, X. Shen, Y. Li, Y. Pan, D. Wang, K. Zheng, A. R. Boccaccini and J. Wei, *Appl. Mater. Today*, 2020, **18**, 100523.
56 Y. Dai, H. Guo, L. Chu, Z. He, M. Wang, S. Zhang and X. Shang, *J. Orthop. Transl.*, 2020, **24**, 198–208.
57 S. N. Sethu, S. Namashivayam, S. Devendran, S. Nagarajan, W.-B. Tsai, S. Narashiman, M. Ramachandran and M. Ambigapathi, *Int. J. Biol. Macromol.*, 2017, **98**, 67–74.
58 J. Y. Oh, G. Yang, E. Choi and J.-H. Ryu, *Biomater. Sci.*, 2022, **10**, 1448–1455.
59 S. S. Lee, L. Laganenka, X. Du, W.-D. Hardt and S. J. Ferguson, *Front. Bioeng. Biotechnol.*, 2021, **9**(794586).
60 B. S. Bal and M. N. Rahaman, *Acta Biomater.*, 2012, **8**, 2889–2898.
61 M. Rahaman and W. Xiao, *Int. J. Appl. Ceram. Technol.*, 2018, **15**, 861–872.
62 M. Mazzocchi, D. Gardini, P. L. Traverso, M. G. Faga and A. Bellosi, *J. Mater. Sci.: Mater. Med.*, 2008, **19**, 2889–2901.
63 M. P. Arts, J. F. C. Wolfs and T. P. Corbin, *Eur. Spine J.*, 2017, **26**, 2372–2379.
64 R. F. M. R. Kersten, F. C. Oner, M. P. Arts, M. Mitroiu, K. C. B. Roes, A. de Gast and S. M. van Gaalen, *Global Spine J.*, 2022, **12**, 1687–1695.
65 B. S. Bal, A. Khandkar, R. Lakshminarayanan, I. Clarke, A. A. Hoffman and M. N. Rahaman, *J. Arthroplasty*, 2009, **24**, 110–116.
66 X. Kong, X. Hu and W. Chai, *J. Orthop. Surg. Res.*, 2022, **17**, 87.
67 C. Skjoldebrand, J. L. Tipper, P. Hatto, M. Bryant, R. M. Hall and C. Persson, *Mater. Today Bio*, 2022, **15**(100270).
68 S. S. Lee, S. Huber and S. J. Ferguson, *Mater. Sci. Eng., C*, 2021, **127**, 112251.
69 Z. Badran, X. Struillou, F. J. Hughes, A. Soueidan, A. Hoornaert and M. Ide, *J. Oral Implantol.*, 2017, **43**, 240–244.
70 Z. Ozkurt and E. Kazazoglu, *J. Oral Implantol.*, 2011, **37**, 367–376.
71 T. Traini, R. Sorrentino, E. Gherlone, F. Perfetti, P. Bollero and F. Zarone, *J. Oral Implantol.*, 2015, **41**, 352–359.
72 R. Zou, L. Bi, Y. Huang, Y. Wang, Y. Wang, L. Li, J. Liu, L. Feng, X. Jiang and B. Deng, *J. Mech. Behav. Biomed. Mater.*, 2023, **141**(105756).
73 A. Salhotra, H. N. Shah, B. Levi and M. T. Longaker, *Nat. Rev. Mol. Cell Biol.*, 2020, **21**, 696–711.
74 M. Zanocco, E. Marin, A. Rondinella, F. Boschetto, S. Horiguchi, W. Zhu, B. J. McEntire, R. M. Bock, B. S. Bal and G. Pezzotti, *Mater. Sci. Eng., C*, 2019, **105**, 110053.
75 A. Sohrabi, C. Holland, R. Kue, D. Nagle, D. S. Hungerford and C. G. Frondoza, *J. Biomed. Mater. Res.*, 2000, **50**, 43–49.
76 G. Pezzotti, N. Oba, W. Zhu, E. Marin, A. Rondinella, F. Boschetto, B. McEntire, K. Yamamoto and B. S. Bal, *Acta Biomater.*, 2017, **64**, 411–420.
77 G. Pezzotti, R. M. Bock, B. J. McEntire, E. Jones, M. Boffelli, W. Zhu, G. Baggio, F. Boschetto, L. Puppulin, T. Adachi, T. Yamamoto, N. Kanamura, Y. Marunaka and B. S. Bal, *Langmuir*, 2016, **32**, 3024–3035.
78 M. Saura, C. Tarin and C. Zaragoza, *Sci. World J.*, 2010, **10**, 624–632.
79 M. Abdolahi, A. Jafarieh, P. Sarraf, M. Sedighiyan, A. Yousefi, A. Tafakhori, H. Abdollahi, F. Salehinia and M. Djalali, *Endocr., Metab. Immune Disord.: Drug Targets*, 2019, **19**, 874–884.
80 N. Basso and J. N. M. Heersche, *Bone*, 2006, **39**, 807–814.
81 C. Pilbeam, in *Bone Regulators and Osteoporosis Therapy*, ed. P. H. Stern, Springer International Publishing, Cham, 2019, vol. 262, pp. 157–175.
82 H. Kalyanaraman, N. Schall and R. B. Pilz, *Nitric Oxide*, 2018, **76**, 62–70.
83 V. Goëb and T. Anne-Priscille, *CIA*, 2010, 345.
84 H. Kalyanaraman, G. Ramdani, J. Joshua, N. Schall, G. R. Boss, E. Cory, R. L. Sah, D. E. Casteel and R. B. Pilz, *J. Bone Miner. Res.*, 2017, **32**, 46–59.
85 M. Ivanova, J. Dao, L. Noll, J. Fikry and O. Goker-Alpan, *J. Clin. Med.*, 2021, **10**(2217).
86 K. Kaneko, Y. Miyamoto, R. Tsukuura, K. Sasa, T. Akaike, S. Fujii, K. Yoshimura, K. Nagayama, M. Hoshino, S. Inoue, K. Maki, K. Baba, D. Chikazu and R. Kamijo, *Nitric Oxide*, 2018, **72**, 46–51.
87 D. Glick, S. Barth and K. F. Macleod, *J. Pathol.*, 2010, **221**, 3–12.
88 I. M. Shapiro, R. Layfield, M. Lotz, C. Settembre and C. Whitehouse, *Autophagy*, 2014, **10**, 7–19.
89 Y. Tang, Y. Mo, D. Xin, Z. Xiong, L. Zeng, G. Luo and Y. Cao, *J. Orthop. Surg. Res.*, 2021, **16**, 719.
90 X. Tong, J. Gu, R. Song, D. Wang, Z. Sun, C. Sui, C. Zhang, X. Liu, J. Bian and Z. Liu, *J. Cell. Biochem.*, 2019, **120**, 1630–1642.

91 H. Chi, M. Kong, G. Jiao, W. Wu, H. Zhou, L. Chen, Y. Qiao, H. Wang, W. Ma and Y. Chen, *J. Biomater. Appl.*, 2019, **34**, 94–103.
92 W. Ruolan, C. Liangjiao and S. Longquan, *J. Nanobiotechnol.*, 2020, **18**, 119.
93 C. Yue, H. Jin, X. Zhang, W. Li, D. Wang, P. Tong, Y. Liu and Z. Tan, *J. Cell. Mol. Med.*, 2021, **25**, 10175–10184.
94 T. Sun, M. Wang, Y. Shao, L. Wang and Y. Zhu, *Biol. Trace Elem. Res.*, 2018, **181**, 82–94.
95 H. Zhou, G. Jiao, M. Dong, H. Chi, H. Wang, W. Wu, H. Liu, S. Ren, M. Kong, C. Li, L. Zhang and Y. Chen, *Biol. Trace Elem. Res.*, 2019, **190**, 327–335.
96 Y. You, W. Ma, F. Wang, G. Jiao, L. Zhang, H. Zhou, W. Wu, H. Wang and Y. Chen, *Life Sci.*, 2021, **264**, 118680.
97 S. Wang, Z. Deng, Y. Ma, J. Jin, F. Qi, S. Li, C. Liu, F.-J. Lyu and Q. Zheng, *Int. J. Biol. Sci.*, 2020, **16**, 2675–2691.
98 C. M. Karner and F. Long, *Bone*, 2018, **115**, 2–7.
99 Y. Wu, M. Wang, H. Feng, Y. Peng, J. Sun, X. Qu and C. Li, *Mol. Cell. Endocrinol.*, 2017, **452**, 84–92.
100 Z. Jin, J. Kho, B. Dawson, M.-M. Jiang, Y. Chen, S. Ali, L. C. Burrage, M. Grover, D. J. Palmer, D. L. Turner, P. Ng, S. C. S. Nagamani and B. Lee, *J. Clin. Invest.*, 2021, **131**, e138935.
101 T. Hasegawa, H. Hongo, T. Yamamoto, M. Abe, H. Yoshino, M. Haraguchi-Kitakamae, H. Ishizu, T. Shimizu, N. Iwasaki and N. Amizuka, *Int. J. Mol. Sci.*, 2022, **23**(9941).
102 W. J. Lee, S. Tateya, A. M. Cheng, N. Rizzo-DeLeon, N. F. Wang, P. Handa, C. L. Wilson, A. W. Clowes, I. R. Sweet, K. Bomsztyk, M. W. Schwartz and F. Kim, *Diabetes*, 2015, **64**, 2836–2846.
103 G. Pezzotti, B. J. McEntire, R. Bock, W. Zhu, F. Boschetto, A. Rondinella, E. Marin, Y. Marunaka, T. Adachi, T. Yamamoto, N. Kanamura and B. S. Bal, *ACS Biomater. Sci. Eng.*, 2016, **2**, 1121–1134.
104 J. Lee, H. Byun, S. K. M. Perikamana, S. Lee and H. Shin, *Adv. Healthcare Mater.*, 2019, **8**, e1801106.
105 Y. Sun, J. Li, X. Xie, F. Gu, Z. Sui, K. Zhang and T. Yu, *Front. Immunol.*, 2021, **12**, 778078.
106 W. J. Lee, S. Tateya, A. M. Cheng, N. Rizzo-DeLeon, N. F. Wang, P. Handa, C. L. Wilson, A. W. Clowes, I. R. Sweet, K. Bomsztyk, M. W. Schwartz and F. Kim, *Diabetes*, 2015, **64**, 2836–2846.
107 J. M. Sadowska and M.-P. Ginebra, *J. Mater. Chem. B*, 2020, **8**, 9404–9427.
108 P. Zhou, D. Xia, Z. Ni, T. Ou, Y. Wang, H. Zhang, L. Mao, K. Lin, S. Xu and J. Liu, *Bioact. Mater.*, 2021, **6**, 810–822.
109 H. Li, W. Wang and J. Chang, *Regener. Biomater.*, 2021, **8**, rbab056.
110 Y. Huang, C. Wu, X. Zhang, J. Chang and K. Dai, *Acta Biomater.*, 2018, **66**, 81–92.
111 C. Magnusson, P. Uribe, R. Jugdaohsingh, J. J. Powell, A. Johansson and M. Ransjö, *J. Biomed. Mater. Res.*, 2021, **109**, 1967–1978.
112 G. Pezzotti, B. J. McEntire, R. Bock, W. Zhu, F. Boschetto, A. Rondinella, E. Marin, Y. Marunaka, T. Adachi, T. Yamamoto, N. Kanamura and B. S. Bal, *ACS Biomater. Sci. Eng.*, 2016, **2**, 1121–1134.
113 M. Lee, K. Rey, K. Besler, C. Wang and J. Choy, in *Macrophages*, ed. M. Kloc, Springer International Publishing, Cham, 2017, vol. 62, pp. 181–207.
114 G. Pezzotti, *Mater. Today Chem.*, 2018, **9**, 80–90.
115 W. Li, G. Ren, Y. Huang, J. Su, Y. Han, J. Li, X. Chen, K. Cao, Q. Chen, P. Shou, L. Zhang, Z.-R. Yuan, A. I. Roberts, S. Shi, A. D. Le and Y. Shi, *Cell Death Differ.*, 2012, **19**, 1505–1513.
116 T. Chino, K. E. Draves and E. A. Clark, *J. Leukocyte Biol.*, 2009, **86**, 933–940.
117 M. C. Walsh and Y. Choi, *J. Bone Miner. Metab.*, 2021, **39**, 54–63.
118 N. Dirckx, M. Van Hul and C. Maes, *Birth Defects Res., Part C*, 2013, **99**, 170–191.
119 K. Dashnyam, G.-Z. Jin, J.-H. Kim, R. Perez, J.-H. Jang and H.-W. Kim, *Biomaterials*, 2017, **116**, 145–157.
120 H. Li, K. Xue, N. Kong, K. Liu and J. Chang, *Biomaterials*, 2014, **35**, 3803–3818.
121 H. Li and J. Chang, *Acta Biomater.*, 2013, **9**, 6981–6991.
122 C. Wang, K. Lin, J. Chang and J. Sun, *Biomaterials*, 2013, **34**, 64–77.
123 J. P. Cooke, *Atheroscler. Suppl.*, 2003, **4**, 53–60.
124 M. Russwurm, C. Russwurm, D. Koesling and E. Mergia, *Methods Mol. Biol.*, 2013, **1020**, 1–16.
125 A. Aicher, C. Heeschen, S. Feil, F. Hofmann, M. E. Mendelsohn, R. Feil and S. Dimmeler, *PLoS One*, 2009, **4**, e4879.
126 S. Guo, X. Zhu, M. Li, L. Shi, J. L. T. Ong, D. Jańczewski and K. G. Neoh, *ACS Appl. Mater. Interfaces*, 2016, **8**, 30552–30563.
127 R. A. Gittens, R. Olivares-Navarrete, A. Cheng, D. M. Anderson, T. McLachlan, I. Stephan, J. Geis-Gerstorfer, K. H. Sandhage, A. G. Fedorov, F. Rupp, B. D. Boyan, R. Tannenbaum and Z. Schwartz, *Acta Biomater.*, 2013, **9**, 6268–6277.
128 M. Padial-Molina, P. Galindo-Moreno, J. E. Fernández-Barbero, F. O'Valle, A. B. Jódar-Reyes, J. L. Ortega-Vinuesa and P. J. Ramón-Torregrosa, *Acta Biomater.*, 2011, **7**, 771–778.
129 I. Marquetti and S. Desai, *Chem. Phys. Lett.*, 2018, **706**, 285–294.
130 Y. Masuda, W. Inami, A. Miyakawa and Y. Kawata, *World J. Microbiol. Biotechnol.*, 2015, **31**, 1977–1982.
131 S. Spriano, V. S. Chandra, A. Cochis, F. Uberti, L. Rimondini, E. Bertone, A. Vitale, C. Scolaro, M. Ferrari, F. Cirisano, G. Gautier di Confiengo and S. Ferraris, *Mater. Sci. Eng., C*, 2017, **74**, 542–555.
132 B. Qiu, M. Li, B. Xu, P. Liu, Q. Chen, B. Xu and Z. Han, *Ceram. Int.*, 2019, **45**, 4268–4273.
133 L. Hao, H. Yang, C. Du, X. Fu, N. Zhao, S. Xu, F. Cui, C. Mao and Y. Wang, *J. Mater. Chem. B*, 2014, **2**, 4794–4801.
134 R. M. Bock, B. J. McEntire, B. S. Bal, M. N. Rahaman, M. Boffelli and G. Pezzotti, *Acta Biomater.*, 2015, **26**, 318–330.

135 K. Rabel, R.-J. Kohal, T. Steinberg, P. Tomakidi, B. Rolauffs, E. Adolfsson, P. Palmero, T. Fürderer and B. Altmann, *Sci. Rep.*, 2020, **10**, 12810.
136 D. D. Bosshardt, V. Chappuis and D. Buser, *Periodontol. 2000*, 2017, **73**, 22–40.
137 F. Mussano, T. Genova, P. Rivolo, P. Mandracci, L. Munaron, M. Faga and S. Carossa, *J. Mater. Sci.*, 2017, **52**, 467–477.
138 T. C. von Erlach, S. Bertazzo, M. A. Wozniak, C.-M. Horejs, S. A. Maynard, S. Attwood, B. K. Robinson, H. Autefage, C. Kallepitis, A. Del Río Hernández, C. S. Chen, S. Goldoni and M. M. Stevens, *Nat. Mater.*, 2018, **17**, 237–242.
139 F. Boschetto, E. Marin, E. Ohgitani, T. Adachi, M. Zanocco, S. Horiguchi, W. Zhu, B. J. McEntire, O. Mazda, B. S. Bal and G. Pezzotti, *Biomed. Mater.*, 2021, **16**, 015015.
140 M. Ishikawa, K. L. de Mesy Bentley, B. J. McEntire, B. S. Bal, E. M. Schwarz and C. Xie, *J. Biomed. Mater. Res.*, 2017, **105**, 3413–3421.
141 V. Edachery, R. Shashank and S. V. Kailas, *Tribol. Int.*, 2021, **158**, 106932.
142 G. Pezzotti, B. J. McEntire, R. Bock, M. Boffelli, W. Zhu, E. Vitale, L. Puppulin, T. Adachi, T. Yamamoto, N. Kanamura and B. S. Bal, *Sci. Rep.*, 2016, **6**, 31717.
143 E. Marin, T. Adachi, M. Zanocco, F. Boschetto, A. Rondinella, W. Zhu, S. Somekawa, R. Ashida, R. M. Bock, B. J. McEntire, B. S. Bal, O. Mazda and G. Pezzotti, *Mater. Sci. Eng., C*, 2020, **106**, 110278.
144 S. R. K. Akin, C. B. Garcia and T. J. Webster, *Ceram. Int.*, 2021, **47**, 1837–1843.
145 J. Raphel, M. Holodniy, S. B. Goodman and S. C. Heilshorn, *Biomaterials*, 2016, **84**, 301–314.
146 W. Zhang, J. Liu, H. Shi, K. Yang, P. Wang, G. Wang, N. Liu, H. Wang, J. Ji and P. K. Chu, *Colloids Surf., B*, 2016, **148**, 354–362.
147 W. Zhang, N. Liu, H. Shi, J. Liu, L. Shi, B. Zhang, H. Wang, J. Ji and P. K. Chu, *Sci. Rep.*, 2015, **5**, 9369.
148 L. Shi, W. Zhang, K. Yang, H. Shi, D. Li, J. Liu, J. Ji and P. K. Chu, *J. Mater. Chem. B*, 2015, **3**, 733–737.
149 X. Zhou, F. M. Moussa, S. Mankoci, P. Ustriyana, N. Zhang, S. Abdelmagid, J. Molenda, W. L. Murphy, F. F. Safadi and N. Sahai, *Acta Biomater.*, 2016, **39**, 192–202.