A mini review focused on the proangiogenic role of silicate ions released from silicon-containing biomaterials

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Abstract
Angiogenesis is considered an important issue in the development of biomaterials for the successful regeneration of tissues including bone. While growth factors are commonly used with biomaterials to promote angiogenesis, some ions released from biomaterials can also contribute to angiogenic events. Many silica-based biomaterials have been widely used for the repair and regeneration of tissues, mainly hard tissues such as bone and tooth structure. They have shown excellent performance in bone formation by stimulating angiogenesis. The release of silicate and others (Co and Cu ions) has therefore been implicated to play critical roles in the angiogenesis process. In this short review, we highlight the in vitro and in vivo findings of angiogenesis (and the related bone formation) stimulated by the various types of silicon-containing biomaterials where silicate ions released might play essential roles. We discuss further the possible molecular mechanisms underlying in the ion-induced angiogenic events.

Keywords
Angiogenesis, silicate ions, silicon-containing biomaterials, bone stimulation

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Introduction

A challenging issue in bone tissue engineering is the development of angiogenic/osteogenic biomaterials which can effectively promote both bone formation and vascularization after implantation. During bone regeneration process, endothelial progenitor and stem cells are initially stimulated to enable angiogenesis, with the secretion of angiogenic factors; also, the stem cells are further committed to osteogenic differentiation and maturation to form vascularized bone structure. Therefore, biomaterials that are developed for bone regeneration should initially stimulate endothelial cells to form vascular networks in the damaged area and then stimulate osteoprogenitor or bone-forming cells to synthesize the extracellular matrix for bone.

Much effort has been exerted to incorporate growth factors within biomaterials such as vascular endothelial growth factor (VEGF) and bone morphogenetic proteins (BMPs) to enhance the angiogenesis and osteogenesis, respectively. For example, the loading of VEGF to poly(lactic-co-glycolic acid) (PLGA) scaffolds significantly stimulated blood vessel formation and bone regeneration in irradiated osseous defects. However, the growth factor approach holds several drawbacks such as high cost, denaturation, or degradation of protein structure during scaffold fabrication and adverse side effects when overdosed.

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As another category of therapeutic molecules, ions have also been incorporated within biomaterials, and the ions used thus far include cobalt, copper, silver, strontium, zinc, iron, phosphate, and silicate.\(^{3,10,11}\) This ionic approach has several advantages, including simplicity, low cost, stability during scaffold processing, and effectiveness at low concentrations. In fact, many different forms of biomaterials have been developed to incorporate those ions for diverse biological effects, such as angiogenesis, osteogenesis, or antibacterial activity.\(^{3,11-13}\)

Among the biomaterials that can incorporate ions, silica-based bioactive glasses (SBGs) have been widely explored for the repair and regeneration of hard and soft tissues.\(^{12,14}\) SBGs can induce the deposition of a mineral phase onto the surface which mimics the inorganic mineral of natural bone. Moreover, they have the ability to induce vascular network formation without utilizing vascular-stimulating growth factors,\(^{12,15}\) thus enhancing the tissue repair processes.\(^{10,12,16}\) Early findings showed that 45S5 Bioglass® particles enhanced vessel formation by stimulating the secretion of angiogenic growth factors such as VEGF and basic fibroblast growth factor (bFGF) from endothelial cells,\(^{17,18}\) which was ultimately helpful for bone formation. Similarly, calcium silicate (CS) bioceramics releasing silicate ions also showed significantly stimulated angiogenesis through the upregulated VEGF in endothelial cells and the activated kinase insert domain receptor (KDR).\(^{3,14}\)

As witnessed in the previous studies, many silicon-containing biomaterials have the potential for bone regeneration through the angiogenic stimulation, and the role of silicate ions in the events can thus be envisaged. This mini review focuses on the silicon-containing biomaterials and highlights their ability to release silicate ions at various concentration levels and the biological role in promoting angiogenesis. We scrutinize the recent in vitro and in vivo findings related to silicate ionic roles and discuss possible mechanisms in the angiogenic stimulation.

**Silicon-containing biomaterials**

Silicate ions that can release during the degradation of silica-based biomaterials (glasses, crystalline ceramics, or composites/hybrids) have been found to influence cell functions and to accelerate osteogenesis and angiogenesis.\(^{10,12}\) Silicate ions release at different levels due to the different degradation rates of biomaterials which depend on the composition, size, and shape.\(^{16}\) A diverse array of silica-networked, silicon-substituted, or silicon-incorporating biomaterials has thus been developed with different forms, such as monoliths/solid blocks, porous scaffolds, nano/microfibers, and nano/microparticles, as presented in Figure 1. In the following sections, these biomaterials are described in detail.

**Glasses**

SBGs are amorphous materials that contain SiO₂ as the main glass network former. The most well-known SBG in biomedical fields is 45S5 Bioglass which has a bioactive and biodegradable silicate composition (see Table 1). This was discovered by Hench\(^{19}\) and Jones and Hench\(^{20}\) in 1969, and since then, many bioactive glass/glass ceramics (melt or sol-gel derived) have been introduced for bone repair and grafting.\(^{21}\) The main characteristic of the bioactive glasses is the well-known ionic reactions and the subsequent formation of hydroxyapatite (HA) layer on their surface in contact with physiological body fluid.\(^{22,23}\) The apatite layer is very similar to that of bone mineral.\(^{19,22,23}\) Furthermore, the dissolved silicate ions from bioactive glasses have stimulating effects on osteoblasts, regulating the expression of several genes including key osteoblastic markers and extracellular matrix proteins.

Xynos et al. studied the effects of ions extracted from Bioglass 45S5 on cell behaviors. A twofold increase in VEGF expression was observed when human primary osteoblasts were treated with the ionic products.\(^{24}\) Although silicate ions could be released to a level of 16.58 ppm after 24 h of incubation, calcium and phosphorus ions were also contained in the treated medium, which might complicate the interpretation of the exact role of silicate ions, which requires further studies.

The release rate of silicate ions varies according to the material shape (spheres, fibers, or irregular), size (micro or nano), and texture (porous or non-porous), which are related to surface area.\(^{25,26,27}\) For example, the dissolution profiles of silicate ions from powder, foam, and monolithic disk of 58S bioactive glass (60 mol% SiO₂, 36 mol% CaO, 4 mol% P₂O₅) with the same mass was compared in simulated body fluid (SBF) for 24 h.\(^{28}\) The results showed that the release of silicate ions was highest for powder (~2.5 mM) and lowest for monolith (~0.178 mM),\(^{14}\) reflecting the role of surface area that is in contact with the medium. In another example, the silicate ions released from irregular bioactive glass (IBG) particles and bioactive glass microspheres (BGMs) showed significantly different release kinetics.\(^{29}\)

Another type of glasses that contain silicon is the calcium phosphate glasses (CPGs). CPGs are biodegradable materials, and their degradation rate is tailored by the compositional change.\(^{10}\) The incorporation of silica in the glass reduced degradation rate,\(^{10}\) and the released silicate ions were shown to regulate key angiogenic factors.

**Crystalline ceramics**

Some bioceramics, for example, akermanite, bredigite, and diopside, are silicate crystallines and thus can release silicate ions in aqueous environments. Akermanite (Ca₄MgSi₂O₇)
has been shown to be bioactive and degradable, releasing silicate ions. Bredigite (Ca$_7$MgSi$_4$O$_{16}$) is another bioactive silicate ceramic with excellent biocompatibility and apatite mineralization ability. The ionic products from the bredigite dissolution significantly promoted stem cell growth.

CS bioceramics comprise another unique class of silica-based bioceramics. They have been used for bone repair materials due to bioactivity, degradability, and biocompatibility. Several studies have reported that CS in powders or coatings exhibited excellent in vitro and in vivo bioactivity. There are three kinds of CSs with different Ca/Si ratios: monocalcium silicate (CaSiO$_3$), dicalcium silicate (Ca$_2$SiO$_4$), and tricalcium silicate (Ca$_3$SiO$_5$). Monocalcium silicate (MCS) is not hydraulic and has two main forms: low temperature phase, wollastonite (β-CaSiO$_3$), and high temperature phase, pseudowollastonite (α-CaSiO$_3$). Dicalcium silicate and tricalcium silicate can be hydrated and then hardened by mixing with liquid phases. The silicate ions released from CS were shown to provide excellent environment for stem cell proliferation and differentiation toward an osteogenic lineage and also for human umbilical vein endothelial cell (HUVEC) proliferation and angiogenesis.

Calcium phosphate bioceramics (HA and tricalcium phosphate (TCP)) are often doped with silicon. In fact, biological apatites are non-stoichiometric and contain small levels of impurities including silicon. The incorporation of silicon within HA lattice was proposed to improve the bioactivity. Thus, several methods were used to produce silicon-substituted HA (Si-HA) with varying silicon content. Silicon can partially replace phosphate (PO$_4^{3-}$) groups in HA lattice to result in Ca$_{10}$(PO$_4$)$_6$(SiO$_4$)$_x$(OH)$_{2-x}$ and the amount incorporated is limited with ranging from 0.1% to 5% by weight. Silicate ion was also doped to α-TCP (α-Ca$_3$(PO$_4$)$_2$), which is widely used as a powder for calcium phosphate cement (CPC). The α-TCP hydrolyzes in aqueous media to convert into calcium-deficient HA. The Si-doped α-TCP was shown to release silicate ions at ~1.5 mM.

Calcium carbonate (CaCO$_3$) was developed to incorporate silicate species. CaCO$_3$ mineral has three polymorphs, that is, calcite, aragonite, and vaterite. Vaterite is the least thermodynamically stable and thus immediately dissolves, releasing calcium ions, while a part of it

![Figure 1. Various forms of silicon-containing biomaterials that can release silicate ions at proper therapeutic doses (silicate ions are symbolized as Si$^{4+}$ for simplicity).](image-url)
re-crystallizes as the most stable calcite. Vaterite particles containing aminopropyl-functionalized silsesquioxane (SiV, where x = 0, 2.6 and 4.9 wt%) were prepared. Compositions of Si2.6V and Si4.9V were shown to hydrolyze and produce soluble silicate species in the range of ~21–50 mM.

### Composites and hybrids

Hybrids and composites containing silicon have also been developed for improved mechanical and biological responses. The silicon-containing glasses and ceramics were either incorporated within biopolymer matrices or mixed
with other inorganic materials. Composite scaffolds of poly(ε-l-lactic acid) (PDLLA) with bioactive wollastonite could release silicate ions over 3 weeks at levels ~1 mM with 20 wt% wollastonite and ~1.5 mM with 40 wt% wollastonite. Organic/inorganic sol–gel hybrid fibers made of silica/poly(t-lactic acid) (PLLA) were also developed. The hybrids with 10% and 20% silica released silicate ions ~5 and ~9 ppm, respectively, within a week. Furthermore, when calcium was also incorporated within the hybrids, the release of silicate ions was observed to increase to ~20 ppm.

Nanocomposite fibrous membranes made of polycaprolactone–gelatin (PCL-GEL, 1:1 ratio) and bioactive glass nanoparticles (BGn, 75%SiO2-25%CaO) were also developed. The scaffolds (CaO:SiO2 = 1.6) could release silicate ions about 57 and 72 ppm, respectively, for 24 and 72 h of immersion in SBF at 37°C. However, a further increase in immersion time up to 21 days showed an almost saturation. CPC composites based on α-TCP were recently prepared with the incorporation of BGn (85%SiO2-15%CaO) up to 10 wt%. The release of silicate ions from the nanocomposite cement was ~2 mM after 2 weeks of immersion in deionized water (pH: 7.4, 37°C).

Effects of Si ion release on angiogenic events (in vitro and in vivo findings)

The in vitro and in vivo findings demonstrated that silicon-containing biomaterials were excellent in promoting angiogenic events, such as endothelial (progenitor) cell homing, cell polarization, migration, angiogenic differentiation, and neo-blood vessel sprouting (as depicted in Figure 2).

**In vitro angiogenic events (migration, gene stimulation, tubule-like formation)**

Endothelial cell migration is a key attribute of the angiogenesis event. The cell migration is driven by three main routes: chemotaxis, haptotaxis, and mechanotaxis. Biomaterials that release ions like silicon can encourage the chemotaxis of cells, enabling a directional migration of cells toward a gradient of chemotactic ions. Generally, the cell migration studies use a transwell membrane where the cells contained in the membrane are to sense the chemotactic molecules released from the biomaterials placed at the bottom of the culture well. Our recent study used this in vitro model to demonstrate the stimulating effects of silicate ion release from silica microspheres on migration of endothelial cells (HUVECs) toward the silica microspheres. The cell migration motility can also be easily assessed in an in vitro cell scratch model, which is often used to assume the wound repair ability of biomaterials. Two different nanosized bioactive glass compositions, 58S and 80S, could enhance the modeled cellular scratch gap within 24 h, suggesting that the released ions might stimulate cellular motility and movement.

Cellular tubulogenesis assay is a common tool to mimic many stages of in vivo angiogenesis, including cell migration, proliferation, vessel branching, and anastomosis processes. For this assay, HUVECs are supported on Matrigel, and the effects of biomaterials or molecules are
examined on the tubular networking of cells. The nano-sized bioactive glasses that could release silicate ions at 2.87–8.39 µg/mL presented significantly higher number of tubules and more stable tubular structure when compared to the glass-free control group. In the process, key angiogenic factors, such as VEGF, FGF, and endothelial nitric oxide synthase (eNOS), that were known to regulate endothelial cell functions, such as proliferation, migration, and differentiation, were expressed at significantly higher levels.48

Using the model, akermanite bioceramics (containing Ca-, Mg-, and Si) were shown to drive angiogenesis by increasing proangiogenic factors and upregulating downstream signaling molecules.12,13,51,52 The akermanite extracts at 1/256 and 1/64 dilutions presented more numbers of node, circle, and tubules than the TCP extracts and control in the Matrigel assay (Figure 3). At the same time, the proangiogenic genes such as KDR, fibroblast growth factor receptor 1 (FGFR1), and eNOS were highly stimulated. The most effective concentration of silicate ions released from akermanite was found to be in the range 0.6–2 µg mL⁻¹ based on the in vitro tubule networking.

A co-culture system is often used to understand cell-to-cell interactions in the in vitro angiogenesis of cells that mimic better the in vivo multicellular conditions. In an earlier mono-culture study, the silicate ions released from CS induced HUVECs by activating VEGF and its receptor KDR to trigger the angiogenic pathway.14,34 In a co-culture of HUVECs with human dermal fibroblasts (HDFs), interestingly, the silicate ions stimulated the synthesis of abundant VEGF in HDF, which in turn activated the KDR expression in HUVECs, indicating an important role of silicate ions in driving surrounding cells to exert paracrine effects on endothelial functions. A therapeutic range for the silicate ion concentration was proposed to be 0.7–1.8
μg/mL in the co-cultures. Possible cellular mechanisms of the angiogenic stimulation by the released silicate ions will be detailed in section “Proposed mechanisms of silicon ionic roles in angiogenesis.”

In vivo findings

The angiogenesis of silica-based biomaterials has been witnessed in different animal models. The chorioallantoic membrane (CAM) model is considered as a simple and popular assay tool to examine in vivo angiogenesis, which allows a rapid growth of capillary networks in confined conditions where biomaterials come in direct contact. 45S5 Bioglass–based glass–ceramic scaffolds were used to examine the angiogenesis in the CAM model, and results showed significant differences with respect to the control polypropylene scaffold. When the 45S5 Bioglass scaffolds were combined with human adipose tissue–derived stem cells (hASCs), angiogenesis, including number of newly formed blood vessels and tube networks, was substantially induced, and the ions released from the scaffolds were presumed to stimulate the hASC function or to directly induce angiogenesis.

The angiogenesis event is considered critical in the wound healing in vivo as the new blood vessel formation is a key event that can provide nutrients and signaling molecules through vascular networks during the healing process. Therefore, the angiogenic ability of biomaterials is often incurred by the wound healing performance. Several silica-based biomaterials have shown excellent wound healing ability in vivo. It was reported that Bioglass had great potential in an in vivo wound healing model. Although the exact mechanism was not clarified, the ionic release from Bioglass might activate endothelial cells and the consequent wound healing. Several silica-based biomaterials have shown excellent wound healing ability in vivo. It was reported that Bioglass had great potential in an in vivo wound healing model. Although the exact mechanism was not clarified, the ionic release from Bioglass might activate endothelial cells and the consequent wound healing. Several silica-based biomaterials have shown excellent wound healing ability in vivo. It was reported that Bioglass had great potential in an in vivo wound healing model. Although the exact mechanism was not clarified, the ionic release from Bioglass might activate endothelial cells and the consequent wound healing. Several silica-based biomaterials have shown excellent wound healing ability in vivo. It was reported that Bioglass had great potential in an in vivo wound healing model. Although the exact mechanism was not clarified, the ionic release from Bioglass might activate endothelial cells and the consequent wound healing. Several silica-based biomaterials have shown excellent wound healing ability in vivo. It was reported that Bioglass had great potential in an in vivo wound healing model. Although the exact mechanism was not clarified, the ionic release from Bioglass might activate endothelial cells and the consequent wound healing.

Combinatory/synergistic effects with other factors

Ions are often incorporated into the silica-based biomaterials to stimulate angiogenesis. Copper is well known to induce hypoxic-mimic conditions, which is helpful for angiogenesis. The copper-doped CS (Cu-CS) was developed and the effects on angiogenesis were carried out. Results showed that the copper and silicate ions released could regulate the expression of hypoxia-induced factor-1α (HIF-1α), resulting in the VEGF upregulation. The optimal silicate and copper ions in the extracts were found to be 0.6 and 0.7 μg mL⁻¹, respectively; of note, this concentration range is lower than the range known to be effective with single ion, thus suggesting a synergistic role of the dual ions in the angiogenesis.

Our group has recently developed a dual factor (ion plus growth factor) delivering system using sol-gel-derived mesoporous silica microspheres. Along with the silicate ions intrinsically present in the silica microspheres, VEGF was extrinsically loaded onto the mesopores of microspheres. The silicate ions release was modulated at the therapeutic range (a few ppm level per day), while VEGF was released over a couple of weeks continuously. The silicate ions were shown to stabilize HIF-1α expression by blocking prolyl hydroxylase domain (PHD2) enzyme and further upregulate key angiogenic factors such as bFGF, VEGF, their receptors, FGFR and KDR, and eNOS. The VEGF/silicate ion co-delivery could demonstrate a synergism of the
therapeutic molecules, such as endothelial cell migration and tubular formation, and the new blood vessel formation in the in vivo CAM model (Figure 5).

Proposed mechanisms of silicon ionic roles in angiogenesis

Based on recent studies, we summarize the hypothetical key points in which silicon may play roles in promoting angiogenic events in cells.

In hypoxia, cells such as fibroblasts and endothelial cells (“Cell I” in Figure 6) are able to sense reduced oxygen levels and stimulate angiogenesis mainly by the activation of HIF-1. HIF-1, heterodimer transcription factor composed of subunits α and β, regulates the expression of VEGF, which is responsible for the activation of angiogenesis. HIF-1α rapidly stabilizes and accumulates in the cytosol and both subunits, α and β, dimerize to form HIF-1 complex which translocates to the nucleus where it activates the hypoxia response elements (HREs). This activation of HRE is required for the upregulation of VEGF. The VEGF released from cells is recognized by VEGF tyrosine kinase receptors (VEGFRs) in endothelial cells (“Cell II” in Figure 6), activating the signaling cascade of angiogenesis. VEGFR2, known as KDR or fetal liver kinase 1 (Flk1), appears to be the most important receptor in the angiogenesis pathway. HRE is upregulated soon after KDR is activated by VEGF, and the endothelial cells express more VEGF which is then released extracellularly. In the process, autocrine (Cell II to Cell II) and paracrine (Cell I to Cell II) signaling is able to activate the angiogenic pathway. In addition, KDR signaling leads to upregulating eNOS and NO production, which is responsible for the proliferation and migration of endothelial cells. At the same time, VE-cadherin is overexpressed at the endothelial adherens junctions, which is essential for blood vessel formation.

Figure 4. In vivo angiogenesis stimulated by silica-based biomaterials in (a) wound healing model: wound closure at different time points and CD31 staining for the new blood vessels (arrows) at day 14 and in (b) bone defect model: β-TCP and PLDLA/β-CS implantation after 4 weeks, radiographs and Van Gieson’s picrofuchsin staining performed (BV: blood vessel; MC: multinucleate cell, and OB: osteoblast cell). Reprint permission was obtained from Wang et al. and Yu et al.
However, under normoxia conditions, HIF-1α is exposed to the hydroxylation at a proline residue by PHD2 protein, also referred to HIF-prolyl hydroxylase (HPH). This hydroxylation of HIF-1α makes it critical for the interaction with von Hippel–Lindau (VHL) protein, a component of the E3 ubiquitin ligase complex, which, by its ubiquitin ligase activity, ubiquitinizes HIF-1α. Consequently, HIF-1α is degraded at the proteasome, and angiogenesis is inhibited.

Often, the growth factors, such as VEGF, bFGF, or TGF-β, are delivered through biomaterials to induce hypoxic-mediated angiogenic stimulation. Furthermore, ions involved in the stimulation of angiogenic processes (e.g., Cu²⁺, Co²⁺, and Si⁴⁺) are used with biomaterials to mimic this hypoxic condition. As demonstrated, those ions may be involved in the stimulation of HIF-1α through PHD2 inhibition. PHD2 uses oxygen (O₂) and 2-oxoglutarate (2-OG) as co-substrates and iron (Fe(II)) and ascorbic acid (AA) as cofactors; therefore, a lack of any of these will lead to the inactivation of the protein; consequently, HIF-1α rapidly accumulates in the cytosol to induce angiogenic signaling pathway. A recent finding revealed that silicate ions released from pure mesoporous silica microspheres were able to stabilize HIF-1α in the cytosol of endothelial cells.

Up to date, the exact molecular pathway for the inhibition of PHD2 or dehydroxylation of HIF-1α mediated by silicate ions is still unclear; thus, further investigation is needed to confirm precise mechanisms. Here, we presume the silicate interaction mechanisms based on the findings of how other ions interact in the events. Similar to other ions, silicate ions are able to flux into cells through a specific subfamily of aquaporin (AQP10), which is involved not only in the permeability of water molecules but also in the passive transport of small solutes. Specificity for silicate ion is determined by concrete residues (XX/R) in the structure domain according to the recent findings.

Transition metal cations such as cobalt (Co²⁺) and nickel (Ni²⁺) resembling iron (Fe²⁺) can replace it in the iron-binding domain of prolyl hydroxylases; thus, PHD2 is not able to hydroxylate HIF-1α. Besides, cobalt and nickel interact directly with HIF-1α by occupying the VHL-binding domain to inhibit the interaction between VHL and HIF-1α.
hydroxylated HIF-1α. It has been seen that although high levels of reactive oxygen species (ROS) may result in high toxicity in cells and tissues, an initial burst of free radicals is beneficial for the initiation of angiogenesis. Cobalt, nickel, and also copper ions can cause oxidative damage to cells by the generation of ROS. ROS, including peroxides, superoxides, hydroxyl radicals, and singlet oxygen, is produced under normal conditions by cells as products of the oxidative phosphorylation in the mitochondria. Under stress environments, such as ionizing radiation, inflammation, or exposure to metals, ROS generation is enhanced leading to cellular damages. While AA mainly functions as a cofactor of enzymes, it also acts as an antioxidant by preventing the oxidation of molecules during oxidative stress. In this way, AA can reduce Fe(III) into the oxidized iron Fe(II), which is necessary for PHD2 activity. Cobalt and nickel may deplete intracellular AA levels by preventing the entrance into the cell through mechanisms of irreversible degradation of AA to smaller products. Free copper ion can participate in the formation of hydroxyl radicals (OH-) in the presence of AA or superoxide. Although the exact mechanism of silicate ionic interaction with AA still remains unknown, we hypothesize that silicate ions may also interact in a similar way, inhibiting the functions of PHD2 by chelating iron in the binding site of PHD2 and/or HIF-1α or interacting directly or indirectly with AA or even by the generation of ROS. Further studies are needed to clarify how silicate ion affects the PHD2 activity through free radicals and intracellular AA which in turn regulates ROS levels in cells.

One note with regard to the role played by silicon is the possible synergism with other ions. Copper and silicon were shown to have a synergistic effect on the co-cultured fibroblasts and endothelial cells, although silicon appeared to show lower stimulatory effects on HIF-1α expression than copper. It is still not clear how silicon helps copper in the angiogenic stimulation; thus, more studies are needed to elucidate the molecular signaling events of the cooperative roles of silicon and copper and also for the case with other ions, such as cobalt or nickel.

**Conclusion**

Many different forms of silicate-based biomaterials have shown excellent performance in the tissue repair and regeneration processes where angiogenesis is considered a key event. As witnessed in this short review, the release of silicate ions at appropriate levels (a few ppm) is thus believed to play some essential roles in the biological events of these biomaterials. Silicate ion can mimic hypoxic conditions to stimulate angiogenesis pathway by increasing the expression of proangiogenic factors, such as VEGF, FGF, and the receptors in endothelial cells, which in turn upregulates the downstream
cascade of angiogenesis including the stimulation of NO. It is considered that silicate ion may inactivate PHD2 by either directly interacting or indirectly depleting intracellular AA or enhancing ROS generation, all of which possibly contribute to stabilizing HIF-1α and the consequent angiogenic events. Besides, it is needed to pay attention to the cooperative action of silicon with other angiogenic promoting ions. Still more studies are needed to clarify the exact molecular mechanisms in which silicate ion contributes to the angiogenesis of cells.

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References
1. Laschke MW, Harder Y, Amon M, et al. Angiogenesis in tissue engineering: breathing life into constructed tissue substitutes. Tissu Eng 2006; 12: 60913044658052.
2. Shi M, Zhou Y, Shao J, et al. Stimulation of osteogenesis and angiogenesis of hBMSCs by delivering Si ions and functional drug from mesoporous silica nanospheres. Acta Biomater 2015; 21: 178–189.
3. Hoppe A, Güldal NS and Boccaccini AR. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials 2011; 32: 2757–2774.
4. Vallet-Regi M and Ruiz-Hernández E. Bioceramics: from bone regeneration to cancer nanomedicine. Adv Mater 2011; 23: 5177–5218.
5. Andrades JA, Narváez-ledesma L, Cerón-torres L, et al. Bone engineering: a matter of cells, growth factors and biomaterials.
6. Arcos D and Vallet-Regi M. Sol-gel silica-based biomaterials and bone tissue regeneration. Acta Biomater 2010; 6: 2874–2888.
7. Kaigler D, Wang Z, Horger K, et al. VEGF scaffolds enhance angiogenesis and bone regeneration in irradiated osseous defects. J Bone Miner Res 2006; 21: 735–744.
8. Rocha FG, Sundback CA, Krebs NJ, et al. The effect of sustained delivery of vascular endothelial growth factor on angiogenesis in tissue-engineered intestine. Biomater 2008; 29: 2884–2890.
9. Sollier J, Moroni L, Van Blitterswijk C, et al, Critical factors in the design of growth factor releasing scaffolds for cartilage tissue engineering. Expert Opin Drug Deliv 2008; 5: 543–566.
10. Kong N, Lin K, Li H, et al. Synergy effects of copper and silicon ions on stimulation of vascularization by copper-doped calcium silicate. J Mater Chem B 2014; 2: 1100.
11. Bose S, Fielding G, Tarafder S, et al. Understanding of dopant-induced osteogenesis and angiogenesis in calcium phosphate ceramics. Trends Biotechnol 2013; 31: 594–605.
12. Zhai W, Lu H, Chen L, et al. Silicate bioceramics induce angiogenesis during bone regeneration. Acta Biomater 2012; 8: 341–349.
13. Habibovic P and Barralet JE. Bioinorganic and biomaterials: Bone repair. Acta Biomater 2011; 7: 3013–3026.
14. Li H and Chang J. Bioactive silicate materials stimulate angiogenesis in fibroblast and endothelial cell co-culture system through paracrine effect. Acta Biomater 2013; 9: 6981–6991.
15. Wang C, Lin K, Chang J, et al. Osteogenesis and angiogenesis induced by porous β-CaSiO3/PDLGA composite scaffold via activation of AMPK/ERK1/2 and PI3K/Akt pathways. Biomater 2013; 34: 64–77.
16. Rahaman MN, Day DE, Sonny Bal B, et al. Bioactive glass in tissue engineering. Acta Biomater 2011; 7: 2355–2373.
17. Day RM. Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro. Tissue Eng 2005; 11: 768–777.
18. Gorustovich AA, Roether JA and Boccaccini AR. Effect of bioactive glasses on angiogenesis: a review of in vitro and in vivo evidences. Tissue Eng Part B, Rev 2010; 16: 199–207.
19. Hench LL. Bioceramics: from concept to clinic. J Am Ceram Soc 1991; 74: 1487–1510.
20. Jones JR and Hench LL. Effect of surfactant concentration and composition on the structure and properties of sol-gel-derived bioactive glass foam scaffolds for tissue engineering. J Mater Sci 2003; 38: 3783–3790.
21. Fi-qi A, Kim JH and Kim HW. Osteoinductive Fibrous Scaffolds of Biopolymer/Mesoporous Bioactive Glass Nanocarriers with Excellent Bioactivity and Long-Term Delivery of Osteogenic Drug. ACS Appl Mater Interfaces 2015; 1140–1152.
22. Kokubo T. Apatite formation on surfaces of ceramics, metals and polymers in body environment. Acta Mater 1998; 46: 2519–2527.
23. Hench LL. Bioceramics. J Am Ceram Soc 1998; 81: 1705–1728.
24. Xynos ID, Edgar AJ, Buttery LDK, et al. Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis. Biochem Biophys Res Commun 2000; 276: 461–465.
25. Radin S, Chen T and Ducheyne P. The controlled release of drugs from emulsified, sol gel processed silica microspheres. Biomater 2009; 30: 850–858.
26. El-Fiqi A and Kim H-W. Mesoporous bioactive nanocarriers in electrospun biopolymer fibrous scaffolds designed for sequential drug delivery. RSC Adv 2014; 4: 4444–4452.
27. Arcos D and Vallet-Regi M. Sol-gel silica-based biomaterials and bone tissue regeneration. Acta Biomater 2010; 6: 2874–2888.
28. Jones JR, Lee PD and Hench LL. Hierarchical porous materials for tissue engineering. Philos Trans A Math Phys Eng Sci 2006; 364: 263–281.
29. Lei B, Chen X, Han X, et al. Unique physical–chemical, apatite-forming properties and human marrow mesenchymal
stem cells (HMSCs) response of sol–gel bioactive glass microspheres. J Mater Chem 2011; 21: 12725.

30. Thein-Han W and Xu HHK. Prevascularization of a gas-foaming macroporous calcium phosphate cement scaffold via coculture of human umbilical vein endothelial cells and osteoblasts. Tissue Eng Part A 2013; 19: 1675–85.

31. Wu C, Chang J, Zhai W, et al. A novel bioactive porous bredigite (Ca7MgSi4O16) scaffold with biomimetic apatite layer for bone tissue engineering. J Mater Sci: Mater Med 2007; 18: 857–864.

32. Lin K, Xia L, Li H, et al. Enhanced osteoporotic bone regeneration by strontium-substituted calcium silicate bioactive ceramics. Biomaterials 2013; 34: 10028–10042.

33. Saravanan S, Vimalraj S, Vairamani M, et al. Role of mesoporous wollastonite (calcium silicate) in mesenchymal stem cell proliferation and osteoblast differentiation: a cellular and molecular study. J Biomed Nanotechnol 2015; 11: 1124–1138.

34. Li H and Chang J. Stimulation of proangiogenesis by calcium silicate bioactive ceramic. Acta Biomater 2013; 9: 5379–5389.

35. Bigi S, Cantalamessa G, Centamore E, et al. The periadriatic basin (Marche–Abruzzi sector, Central Italy) during the Plio-Pleistocene. Geology 1997; 59: 245–259.

36. Murakami S, Hosono T, Jeyadevan B, et al. Hydrothermal synthesis of magnetite/hydroxyapatite composite material for hyperthermia therapy for bone cancer. J Ceram Soc Japan 2008; 116: 950–954.

37. Palard M, Champion E and Foucaud S. Synthesis of silicated hydroxyapatite Ca10(P04)6-x(SiO4 )x(OH)2-x. J Solid State Chem 2008; 181: 1950–1960.

38. Motisuke M, García-Carrodeguas R, Zavaglia CAC, et al. A comparative study between α-TCP and Si-α-TCP calcium phosphate cement. Key Eng Mater 2009; 396–398: 201–204.

39. Tas AC. Vaterite bioceramics: monodisperse CaCO3 biconvex micropills forming at 70°C in aqueous CaCl2-gelatin-area solutions. 139–151.

40. Nakamura J, Poologasundarampillai G, Jones JR, et al. Tracking the formation of vaterite particles containing silesquioxane and their structure for bone regenerative medicine. J Mater Chem B 2013; 1: 4446.

41. Poologasundarampillai G, Yu B, Jones JR, et al. Electrospun silica/PLLA hybrid materials for skeletal regeneration. Soft Matter 2011; 7: 1024.

42. Li H and Chang J. Preparation and characterization of bioactive and biodegradable Wollastonite/poly(D,L-lactic acid) composite scaffolds. J Mater Sci: Mater Med 2004; 15: 1089–1095.

43. A El-Fiqi JH and Kim HK. Novel bioactive nanocomposite cement formulations with potential properties: incorporation of the nanoparticle form of mesoporous bioactive glass into calcium phosphate cements. J Mater Chem B; 7(2): 1321–1334.

44. Lamalice L, Le Boeuf F and Huot J. Endothelial cell migration during angiogenesis. Circ Res 2007; 100: 782–794.

45. Handel M, Hammer TR, Nooead P, et al. 45S5-Bioglass®-based 3D-scaffolds seeded with human adipose tissue-derived stem cells induce in vivo vascularization in the CAM angiogenesis assay. Tissue Eng Part A 2013; 19: 2703–2712.

46. Dashynam K, Jin G-Z, Kim J-H, et al. Promoting angiogenesis with mesoporous microcarriers through a synergistic action of delivered silicon ion and VEGF. Biomaterials 2017; 116: 145–157.

47. Goodwin AM. NIH Public Access. North 2009; 74: 172–183.

48. Mao C, Chen X, Miao G, et al. Angiogenesis stimulated by novel nanoscale bioactive glasses. Biomed Mater 2015; 10: 25005.

49. Hong JK, Bang JY, Xu G, et al. Thickness-controllable electrospun fibers promote tubular structure formation by endothelial progenitor cells. Int J Nanomedicine 2015; 10: 1189–1200.

50. Goodwin AM. In vitro assays of angiogenesis for assessment of angiogenic and anti-angiogenic agents. Microvasc Res 2007; 74: 172–183.

51. Xia L, Yin Z, Mao L, et al. Akermanite bioceramics promote osteogenesis, angiogenesis and suppress osteoclastic aggregation for osteoporotic bone regeneration. Sci Rep 2016; 6: 22005.

52. Sun H, Wu C, Dai K, et al. Proliferation and osteoblastic differentiation of human bone marrow-derived stromal cells on akermanite-bioactive ceramics. Biomaterials 2006; 27: 5651–5657.

53. Lokman NA, Elder ASF, Ricciardelli C, et al. Chick chorioallantoic membrane (CAM) assay as an in vivo model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. Int J Mol Sci 2012; 13: 9959–9970.

54. Hoeben ANN, Landuyt B, Highley MSM, et al. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004; 56: 549–580.

55. Piraino F and Selimović S. A current view of functional biomaterials for wound care, molecular and cellular therapies. Biomed Res Int 2015; 2015: 1–10.

56. Yu H, Peng J, Xu Y, et al. Bioglass activated skin tissue engineering constructs for wound healing. ACS Appl Mater Interfaces 2016; 8: 703–715.

57. Li H, He J, Yu H, et al. Bioglass promotes wound healing by affecting gap junction connexin 43 mediated endothelial cell behavior. Biomaterials 2016; 84: 64–75.

58. Jabbarzadeh E, Blanchette J, Shazly T, et al. Vascularization of biomaterials for bone tissue engineering: current approaches and major challenges. Curr Angiogenesise 2012; 1: 180–191.

59. Leu A, Stieger SM, Dayton P, et al. Angiogenic response to bioactive glass promotes bone healing in an irradiated calvarial defect. Tissue Eng Part A 2009; 15: 877–885.

60. Juddhaosinh R. Silicon and bone health. J Nutr Heal Aging 2007; 11: 99–110.

61. Carlisle EM. Silicon: a possible factor in bone calcification. Science 1970; 167: 279–280.

62. Wu C and Chang J. Degradation, bioactivity, and cytocompatibility of diopside, akermanite, and bregidite ceramics. J Biomed Res Part B 2007; 83B: 153–160.

63. Wu C, Chang J, Wang J, et al. Preparation and characterisistics of a calcium magnesium silicate (bregidite) bioactive ceramic. Biomaterials 2005; 26: 2925–2931.
64. Lindahl C, Xia W, Lausmaa J, et al. Strontium and silicon co-doped apatite coating: preparation and function as vehicles for ion delivery. J Biomater and Nanobiotechnology 2012; 3, 335–334; 335–341.

65. Mohammadi MS, Ahmed I, Muja N, et al. Effect of Si and Fe doping on calcium phosphate glass fibre reinforced polycaprolactone bone analogous composites. Acta biomaterialia 2012; 4: 1616–1626.

66. Perez RA, Kim JH, Buitrago JO, et al. Novel therapeutic core-shell hydrogel scaffolds with sequential delivery of cobalt and bone morphogenetic protein-2 for synergistic bone regeneration. Acta Biomater 2015; 295–308.

67. Lee SI, Lee ES, El-Fiqi A, et al. Stimulation of odontogenesis and angiogenesis via bioactive nanocomposite calcium phosphate cements through integrin and VEGF signaling pathways. J Biomed Nanotechnol 2016; 12: 1048–1062.

68. Fiocco L, Li S, Bernardo E, et al. Highly porous polymer-derived wollastonite–hydroxy carbonate apatite ceramics for bone regeneration. Biomedical Mater 2016; 11: 25016.

69. Montesano R, Pepper MS and Orci L. Paracrine induction of angiogenesis in vitro by Swiss 3T3 fibroblasts. J Cell Sci 1993; 105(Pt 4): 1013–1024.

70. Johnson KE and Wilgus TA. Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair. Adv Wound Care 2014; 3: 647–661.

71. Maxwell KPS. HIF 1 an oxygen and metal responsive transcription factor. Cancer Biol Ther 2004; 3: 29–35.

72. Fong G-H and Takeda K. Role and regulation of prolyl hydroxylase domain proteins. Cell death differ 2008; 15: 635–641.

73. Giannotta M, Tran M and Dejana E. VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. Dev Cell 2013; 26: 441–454.

74. Garneau AP, Carpentier GA, Marcoux A-A, et al. Aquaporins mediate silicon transport in humans. PLOS ONE 2015; 10: e0136149.

75. Carpentier GA, Garneau AP, Marcoux A-A, et al. Identification of key residues involved in Si transport by the aquaglyceroporins. J Gen Physiol 2016; 148: 239–251.

76. Takata K, Matsuzaki T and Tajika Y. Aquaporins: water channel proteins of the cell membrane. Prog Histochem Cytochem 2004; 39: 1–83.

77. Verkman AS. Mammalian aquaporins: diverse physiological roles and potential clinical significance. Exp Rev Mol Med 2008; 10: e13.

78. Yuan Y, Hilliard G, Ferguson T, et al. Cobalt inhibits the interaction between hypoxia-inducible factor- and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor. J Biol Chem 2003; 278: 15911–15916.

79. Maulik N and Das DK. Redox signaling in vascular angiogenesis. Free Radic Biol Med Radic Biol Med 2002; 33: 1047–1060.

80. Salnikow K, Donald SP, Bruick RK, et al. Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. J Biol Chem 2004; 279: 40337–40344.

81. Gaetke LM and Chow CK. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 2003; 189: 147–163.

82. Jomova K, Baros S and Valko M. Redox active metal-induced oxidative stress in biological systems. Transit Met Chem 2012; 37: 127–134.