Bioactive Materials 49 (2025) 271–290

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: www.keaipublishing.com/en/journals/bioactive-materials

Review article

Unraveling osteogenesis mechanisms of the empowered VitaFlux adaptive regeneration biomaterials for bone tissue engineering: Insights into the role of BBGs/BSBGs

Xian Li [a,1], Kun Su [a,1], Limin Zhao [b,c,1], Hao Zhang [a], Qiang Yang [d], Ping Du [a,*], Xiaofeng Chen [e,f,g,**], Haobo Pan [a,b,***]

[a] Shenzhen Key Laboratory of Marine Biomedical Materials, CAS-HK Joint Lab of Biomaterials, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, 518055, PR China
[b] Shenzhen Healthemes Biotechnology Co. Ltd, Shenzhen, 518102, PR China
[c] Geriatric Medicine Department and General Medicine Department, Shenzhen Longhua District Central Hospital, Shenzhen, 518000, PR China
[d] Department of Spine Surgery, Tianjin Hospital, Tianjin University, Tianjin, 300211, PR China
[e] National Engineering Research Center for Tissue Restoration and Reconstruction, Guangzhou, 510006, PR China
[f] Department of Biomedical Engineering, School of Materials Science and Engineering, South China University of Technology, Guangzhou, 510641, PR China
[g] School of Medicine, Foshan University, Foshan, 528000, PR China

ARTICLE INFO

Keywords:
Bone tissue engineering
Bioactive glasses (BGs)
Borate and borosilicate bioactive glasses (BBGs/BSBGs)
Osteogenesis
Alkaline microenvironment
Immunomodulation

ABSTRACT

Bone tissue engineering materials are crucial for bone repair, but existing repair materials still face many challenges, including poor biocompatibility and bioactivity, slow self-repair processes, limited adaptability, inability to promote angiogenesis and so on. To address these issues, the development of third-generation bone repair materials, which are being designed to stimulate specific cellular responses at the molecular level, such as borate and borosilicate bioactive glasses (BBGs/BSBGs) that activate cells and genes, offers new potential for promoting bone tissue self-renewing. Their unique characteristic lies in a flow of life-giving energy, releasing beneficial ions such as boron, calcium and silicon to stimulate cell proliferation and differentiation, accelerating the regeneration of bones. Through this dynamic repair mechanism, these VitaFlux glasses operate like a "living system" within the body, not only speeding up the healing of damaged tissues but also interacting seamlessly with surrounding tissues during the repair process. In this review, we provide a comprehensive analysis of the current understanding of the osteogenesis mechanisms of BBGs/BSBGs, emphasizing their interactions with cells, including ion release and exchange, protein adsorption, and cell adhesion. We also examine key osteogenic signaling pathways related to the alkaline and ionic microenvironments of BBGs/BSBGs, such as the cell cycle, Wnt, MAPK, and BMP signaling pathways, along with macrophage polarization and angiogenesis. Additionally, strategies and future prospects for advancing BBGs/BSBGs research are discussed. Special attention is given to the NaBC1 and GPCR-mediated signaling pathways, which require further investigation.

1. Introduction

Bone tissue engineering biomaterials are vital in bone repair, providing structural support, stabilization, and promoting new bone formation [1,2]. Despite their importance, designing and fabricating these materials remains challenging due to issues such as limited biocompatibility, insufficient bioactivity, slow self-repair mechanisms, poor angiogenesis, and inadequate adaptability. Initially, biomaterials

Peer review under the responsibility of KeAi Communications Co., Ltd.

* Corresponding author. Shenzhen Key Laboratory of Marine Biomedical Materials, CAS-HK Joint Lab of Biomaterials, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, 518055, PR China.
** Corresponding author. National Engineering Research Center for Tissue Restoration and Reconstruction, Guangzhou, 510006, PR China.
*** Corresponding author. Shenzhen Key Laboratory of Marine Biomedical Materials, CAS-HK Joint Lab of Biomaterials, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, 518055, PR China.
E-mail addresses: ping.du@siat.ac.cn (P. Du), chenxf@scut.edu.cn (X. Chen), hb.pan@siat.ac.cn (H. Pan).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bioactmat.2025.03.006
Received 9 November 2024; Received in revised form 7 February 2025; Accepted 6 March 2025

were designed to match the physical properties of replaced tissues while minimizing toxicity (first generation biomaterials) [2–4]. Subsequent focus began to shift to developing bioactive materials that actively interact with physiological environments, eliciting controlled cellular responses. With advancements in science and medicine, expectations for biomaterials have increased, and achieving "perfect" bone repair remains a significant challenge. Third-generation biomaterials, which combine bioactivity with resorbability, aim to stimulate specific cellular responses at the molecular level, maybe potentially overcoming these challenges. Among these advanced biomaterials, cell- and gene-activating bioactive glasses (BGs) also offer innovative approaches for bone repair by stimulating, responding to, and inducing tissue self-repair [1].

Discovered by Professor Larry L. Hench in 1969 [4,5], BGs are inorganic, amorphous biomaterials widely applied in the repair of bone, teeth, and skin [4]. Their unique properties make them indispensable in medical treatments. On one hand, BGs form a hydroxycarbonate apatite (HCA) layer on their surface through mineral deposition in physiological environments [1,6–8], creating strong biological bonds with human tissues [2,4,9]. This ensures excellent biocompatibility, reduces inflammation, minimizes rejection, and accelerates healing [10–12]. On the other hand, BGs release ions that activate specific signaling pathways and downstream genes, promoting tissue regeneration [1,13]. These excellent functions establish BGs as exemplary "third-generation" biomaterials.

BGs are classified based on their primary oxide composition: silicate (SiO_2-based), borate (B_2O_3-based), and phosphate (P_2O_5-based) glasses. Among these, borate and borosilicate bioactive glasses (BBGs/BSBGs) stand out due to their tunable properties. Boron and silicon share similar chemical properties and play essential roles in biological processes. By replacing all or part of the silicon in BGs with boron, borate and borosilicate BGs (BBGs/BSBGs) have been developed [6], expanding their applications and making BBGs/BSBGs valuable tools for tissue engineering. By adjusting boron concentration, BBGs/BSBGs can align their degradation rates and mechanical properties with tissue regeneration needs. Compared to silicate bioactive glasses (SBGs), their structure, composed of boron oxide triangles, grants them higher chemical reactivity compared to silicate glasses. These materials degrade faster in vivo, form hydroxyapatite more readily, and exhibit lower melting temperatures, making them easier to process and shape. Additionally, their ability to incorporate various modifying ions expands their versatility and potential applications.

BBGs/BSBGs exhibit adaptive responsiveness, and dynamically interact with cells and tissues by releasing bioactive ions, such as boron, calcium, and silicon, which stimulate cell proliferation and differentiation to accelerate the regeneration of bone. Meanwhile, they promote angiogenesis, while enhancing blood supply to the repair site. These materials demonstrate exceptional "life activation" capabilities. The dynamic, constantly changing, and self-repairing properties inspired using the term "VitaFlux", symbolizing the flow of life-giving energy. Their remarkable adaptability, bioactivity, and processability continue to attract significant attention, driving advancements in medical and engineering research.

Researchers have extensively studied the fabrication, chemical properties, degradation processes, surface reaction kinetics, and ion doping of BBGs/BSBGs, as well as their various application fields. Additionally, preliminary explorations into their osteogenic mechanisms have been made. This review article will focus on the osteogenesis mechanisms of BBGs/BSBGs at the cellular and molecular levels, delving into the effects of their microenvironment (including ion and pH microenvironment), the activation of related signaling pathways, strategies and future perspectives in the field.

2. Chemical compositions and structural characteristics of BBGs/BSBGs

With advancements in material preparation technology, BGs have seen continuous improvements in both performance and production processes. Based on the preparation processes, the development of BGs can be divided into three stages: melting BGs, sol-gel BGs, and template-assisted sol-gel BGs (Fig. 1 shows three kinds of BGs prepared by our groups by different methods [14–16]). The melt-derived method is straightforward but has drawbacks, such as a dense surface and uneven mixing of the glass. Sol-gel-derived BGs, on the other hand, are formed by the continuous stacking of spherical particles, resulting in micro- and nanopores that provide a larger surface area and enhanced biological activity. In the third generation of BGs, structure-directing agents and templates are introduced into the sol-gel reaction system, forming specific structures through small-molecule self-assembly. This template-assisted sol-gel method successfully overcomes challenges such as uncontrollable morphology and particle size. Several different morphology BGs were produced by our groups with different templates and methods [17–20] Fig. 2). BBGs/BSBGs are primarily produced using these above-mentioned three methods by replacing all or part of the silicon in BGs with boron.

Research on BBGs/BSBGs has made significant progress. However, changes in their composition can have substantial effects on the properties of these BGs, which are closely related to their molecular structures. The molecular network in BBGs is primarily composed of boron-oxygen triangles [BO_3], boron-oxygen tetrahedra [BO_4], and phosphorus-oxygen polyhedra [PO_X]. Boron is typically introduced into BGs in the form of B_2O_3. When boron exists in the [BO_4] coordination, it strengthens the network and enhances the corrosion resistance of the BGs. In contrast, when boron exists as a [BO_3] triangle, the boron oxide trihedron [BO_3] forms a layered structure. These molecular layers are held together by molecular forces, reducing the spatial connectivity of the structure and increasing its chemical reactivity. Similar to SBGs, other elements like Na^+ and Ca^{2+} are incorporated into the gaps of the BBG network. The hydrolysis enthalpy of saturated B_3-O-B_3 in BBGs is −16.98 kJ/mol, and the hydrolysis rate increases with higher B_2O_3 content [21,22]. This indicates that the degradation rate of BBGs, both in vivo and in vitro, can be modulated by adjusting the boron content.

In the network structure of borosilicate bioactive glasses (BSBGs) (Fig. 3 Biomaterial Microenvironment), [BO_3], [BO_4], and [SiO_4] units are connected by "-Si-O-B-" bonds, forming a relatively stable, short-length, and ordered three-dimensional molecular structure [23]. The ionic radius of elements is an important factor influencing the properties of BGs. For example, B^{3+} ions have a small radius, leading to a looser B_2O_3 structure, which allows HCA to form more quickly. The hydrolysis enthalpy of Si_4-O-B_3 is −3.03 kJ/mol, which falls between the enthalpies of Si_4-O-Si_4 (16.61 kJ/mol) and B_3-O-B_3 (−16.98 kJ/mol). As a result, by adjusting the relative content of boron and silicon, the degradation rate of BSBGs can be regulated, optimizing their biological activity [21,22,24,25].

In addition, the system of trigonal planar [BO_3] and tetrahedral [BO_4] units in BBGs/BSBGs is less durable than the tetrahedral SiO_2 units in SBGs due to reduced network connectivity [8,26]. Yao et al. [7] developed a type of double alkali bioactive glass material with the composition $6Na_2O$-$8K_2O$-8MgO-22CaO-$18xB_2O_3$-$(54-18x)SiO_2$-$2P_2O_5$. After crushing and grinding, 150–300 μm BG particles were obtained and categorized into four groups: 0B, 1B, 2B, and 3B, corresponding to x = 0, 1, 2 and 3, respectively. The preparation methods for all groups were identical. When these particles were placed in a diluted K_2HPO_4 solution (pH = 7) at 37 °C, the degradation rate of the BGs was found to be composition-dependent. As the value of x increased from 0 to 3, the material gradually transitioned from SBGs to BSBGs and then to BBGs. The results showed that higher boron content led to a faster degradation rate, indicating that the boron content can be adjusted according to specific needs to control the degradation rate in both experimental and

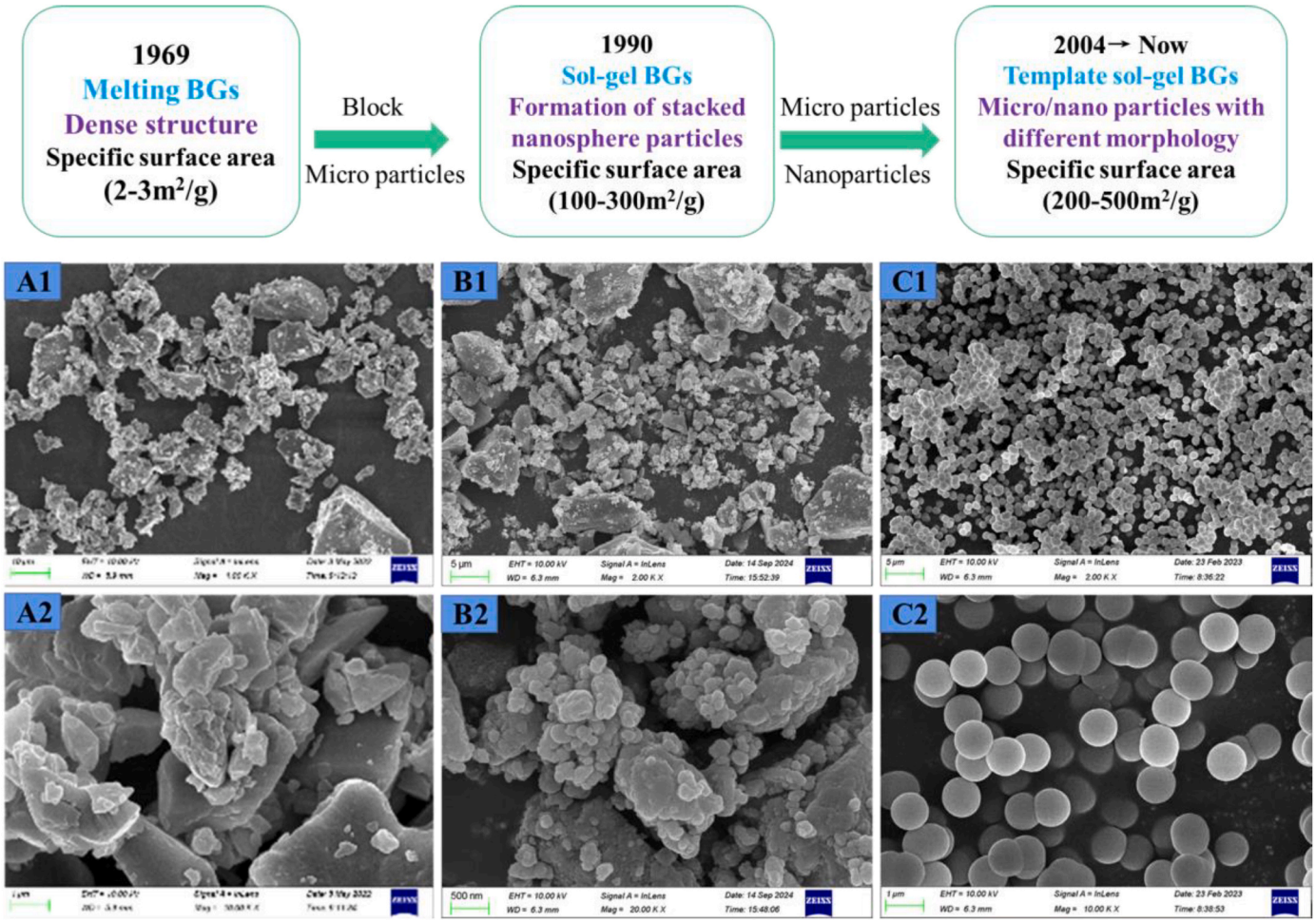

Fig. 1. BGs prepared by different methods.
SEM results of Melting BGs(A1, A2); Sol-gel BGs(B1, B2); Template sol-gel BGs(C1, C2).

clinical applications.

As described above, the structure and composition of BBGs/BSBGs are closely related to their degradation, which affects the rate and type of ion release, and further affects osteogenesis. BBGs/BSBGs have a profound impact on osteogenesis by dynamically interacting with the biological environment. Key bioactive ions such as boron, calcium, and silicon released during the degradation process, each playing a crucial role in bone regeneration. Boron affects receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin expression, regulates mineralized tissue-associated proteins and againsts inflammatory response [27–29], while calcium is vital for mineral deposition and hydroxyapatite formation [30]. Silicon supports collagen synthesis and osteoblast differentiation [31,32]. The unique boron oxide network structure of BBGs/BSBGs leads to faster degradation in physiological conditions, providing a sustained supply of ions, which in turn accelerates bone regeneration and encourages the formation of a hydroxyapatite layer, facilitating direct bonding with bone tissue [1,9]. Additionally, BBGs/BSBGs possess customizable mechanical properties, allowing them to achieve an optimal balance between mechanical strength and biological compatibility. By adjusting boron levels and incorporating other ions, these materials can further enhance specific biological functions, such as improving osteoblast adhesion, promoting protein synthesis, and reducing bone resorption. Their adaptive degradation not only regulates local pH and ionic concentrations, fostering an environment conducive to bone formation, but also supports macrophage polarization towards a pro-regenerative state. This adaptability combines with their ability to stimulate critical signaling pathways such as Wnt/β-catenin and bone morphogenetic protein (BMP). The related mechanisms, osteogenic signaling pathways and direct effects of physicochemical properties of the substrate material's surface will be discussed in the following part "Osteogenesis mechanism".

In addition, in terms of composition, BBGs/BSBGs possess ideal glass-forming regions, making them easy to process and capable of incorporating multifunctional elements. Doped ions play a crucial role in modulating the biological activities of BBGs/BSBGs by influencing their degradation behavior, ion release profiles, and subsequent interactions with the surrounding biological environment. Key doped ions such as magnesium (Mg), zinc (Zn), strontium (Sr), copper (Cu), and silver (Ag) exhibit specific biological functions [1,30,33,34]. Magnesium ions, for instance, are known to enhance osteoblast adhesion and proliferation while also participating in the synthesis of bone matrix proteins and collagen [30,35]. Zinc contributes to enzymatic activity and protein synthesis essential for bone tissue formation [2]. Furthermore, zinc has been shown to exhibit anti-inflammatory and antimicrobial properties, reducing the risk of infection [1,30]. Strontium, another key doped ion, has a dual effect of stimulating osteoblast activity and modulating macrophage responses, which is crucial for maintaining the balance between bone formation and degradation [16,33,36]. Copper ions promote angiogenesis by stimulating endothelial cell proliferation and migration, essential for nutrient delivery to regenerating tissues [33]. Silver ions, on the other hand, exhibit strong antibacterial properties, reducing the risk of infection at the implantation site [30,34]. The ions doped can be purposefully selected like "cocktail" [37] or combined with other treatment according to requirements, providing and

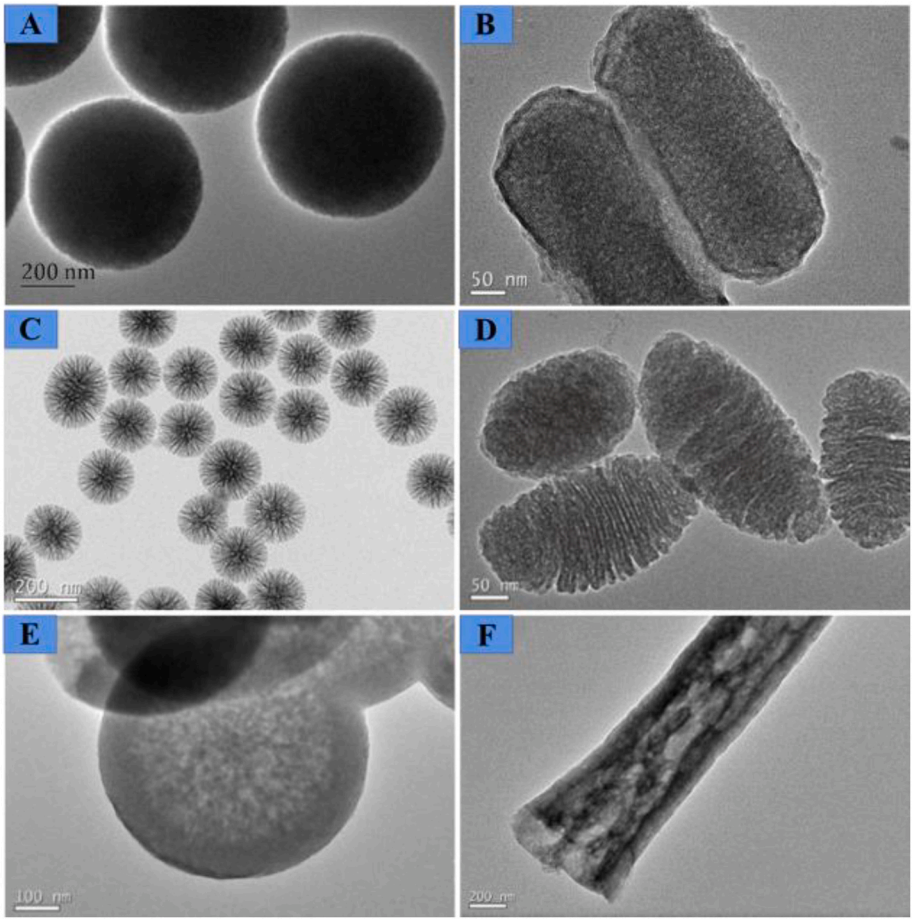

Fig. 2. BGs with different morphology prepared by different methods
(A: Spherical; B: Rod-shaped; C: Radical; D: Pinecone-like [18]; E: Hollow; F: Fibrous).

beneficial for combination strategies in the future directions of biomaterials-based therapies [24,38,39]. The adjustable composition also allows for the fine-tuning of biological effects to achieve optimal material performance. Therefore, when modifying the composition and elemental content in BBGs/BSBGs, it is essential to consider factors such as the ionic radius, acidity, and network binding properties of the elements, especially when doping with multiple elements. This comprehensive approach ensures the desired biological and material properties are achieved.

3. Osteogenesis mechanism

3.1. Ion release, exchange, and hydroxyapatite formation

BBGs and BSBGs have demonstrated the ability of mineralization in vitro in simulated body fluid (SBF) or other fluid [40]. The process of ion release, exchange, and HCA formation in BBGs/BSBGs is similar to that of SBGs. Upon contact with SBF, body fluids, or other phosphorus-containing solutions, a rapid exchange of cations with H^+ ions occurs, causing boron ions to enter the solution as borate groups. This leads to the formation of a boron layer (with or without silanol groups) on the surface of BBGs/BSBGs, providing nucleation sites for calcium to bind with phosphate ions in the solution. An amorphous, calcium phosphate-rich layer forms on top of this boron layer through the incorporation of soluble calcium and phosphate ions from the solution. This accelerates mineralization and deposition by incorporating CO_3^{2-} or other anions from the solution, leading to the formation of an HCA layer (Fig. 3 Mineralization). This HCA layer, formed through in-situ deposition, naturally possesses a porous structure that facilitates the diffusion of Na^+ and Ca^{2+}, further promoting the degradation of BBGs/BSBGs [6] This explains why BBGs/BSBGs degrade faster than SBGs.

3.2. Protein adsorption

With the deepening research in biomaterials, researchers believe that the concept of "bioactivity" not only includes the ability of materials to induce the formation of HCA and form chemical combination with tissues [1,4], but also the properties of materials such as the interaction between materials and cells and the stimulation of special biological reactions of cells should become an important basis for evaluating the bioactivity of materials [41]. Third-generation BGs are being designed to activate genes that stimulate regeneration of living tissues [2]. When biomaterials come into contact with human tissues or cells, they first adsorb water and ions, followed by adhesive proteins from the extracellular matrix (ECM), forming a protein aggregation layer. This layer mediates various biological processes such as cell adhesion, migration, proliferation, and differentiation [42]. The surface properties of the material play a crucial role in regulating protein adsorption, influencing the interaction between the substrate surface and proteins [43]. Protein adsorption is governed by several forces, including attractive Coulomb forces, van der Waals forces, hydrogen bonding, and the entropy increase of solvent molecules or counter-ions between the material surface and specific regions of the protein. These interactions cause the protein to be adsorbed in an orientation that minimizes the total free energy of the system [43]. During this process, proteins may undergo conformational changes, which can affect their orientation and subsequent cell adhesion behavior. The adsorption of proteins onto material surfaces is

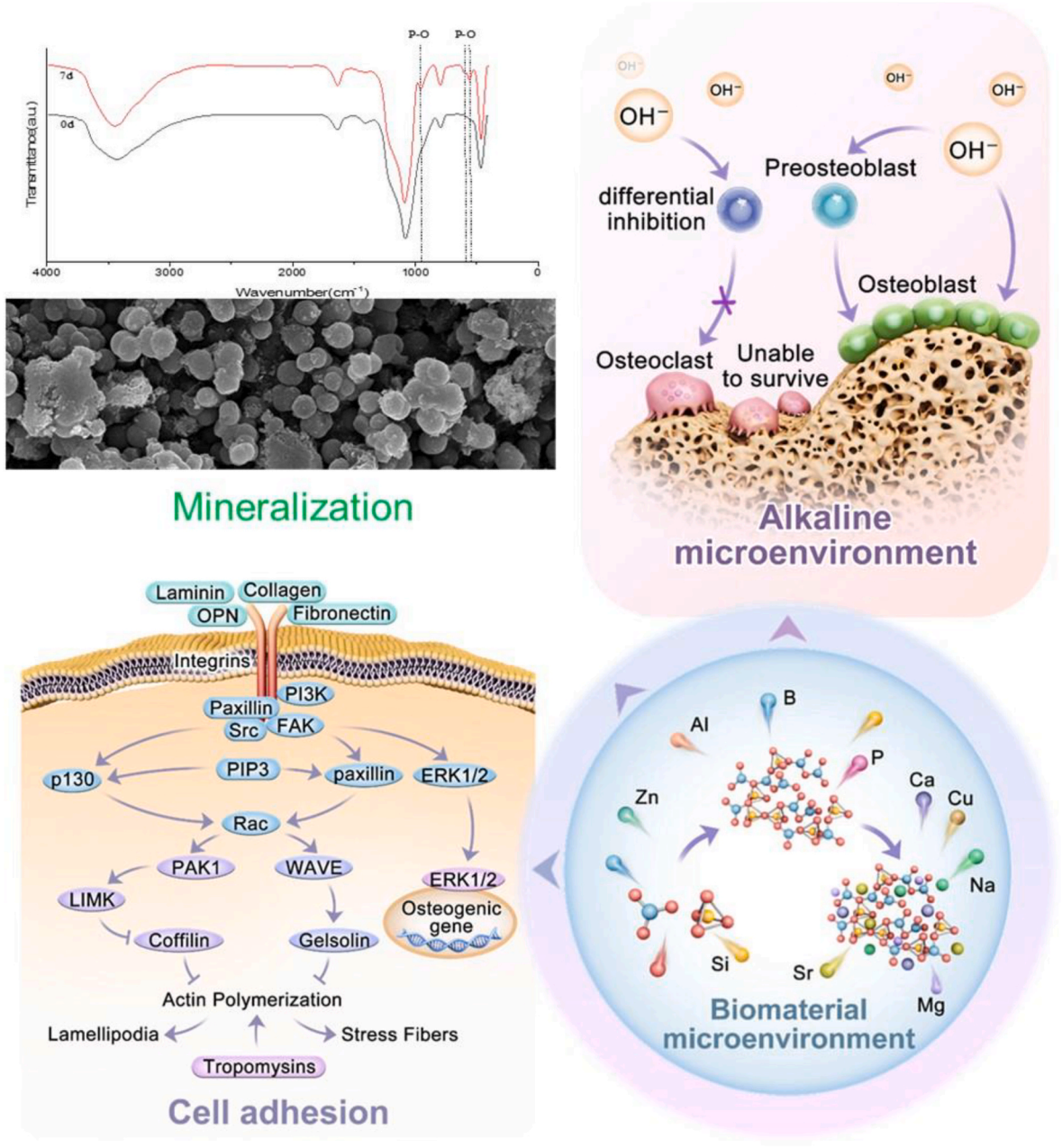

Fig. 3. Biomaterial microenvironment of BBGs/BSBGs regulate osteogenesis through mineralization, cell adhesion and alkaline microenvironment.

the initial step in many biological processes. In particular, the non-specific adsorption of proteins on the surface of tissue engineering biomaterials, especially those in direct contact with blood flow, plays a critical role in their vascularization performance. On the other hand, the adsorption of adhesive proteins onto biomaterial surfaces can modulate the biological response to the materials, making it a key factor in determining their biocompatibility.

The B-rich gel layer (with or without silanol groups) and the HCA layer of BGs possess high surface areas, enabling them to adsorb large quantities of biomolecules, which in turn promotes extracellular responses. BGs can selectively adsorb serum proteins, such as fibrin, and enhance cell adhesion and the expression of osteoblast phenotypes [13,44]. Additionally, BGs can directly facilitate the differentiation of osteoprogenitor cells into osteoblasts, demonstrating strong osteogenic effects [5,45] Notably, BGs are the only known bioceramics that can form strong chemical bonds with both hard and soft tissues [46]. Animal transplantation experiments have further confirmed that BG composites do not induce local or systemic toxicity, reinforcing their safety and biocompatibility.

The physicochemical properties of a substrate material's surface—such as morphology, composition, charge properties, surface energy, polarity [47] and roughness [48]—can significantly influence the interaction between the material and proteins. In a study by Leu et al., five different architectures (cubic, spherical, x, gyroid, and diamond) were fabricated with BBGs at varying porosities. The results demonstrated that BBG scaffolds with a diamond architecture promoted greater fibrous tissue formation in rat calvarial defects, suggesting that these scaffolds hold promise for bone repair applications [49].

Protein adsorption and the formation of the integration interface are closely interconnected. Ions released from Sr-containing BBG particles, such as Ca, P, B, and Sr, contribute to the bioactivity and osseointegration of BBG-poly(methylmethacrylate) (BBG-PMMA) composites, resulting in the promotion of the adhesion, migration, proliferation, and collagen secretion of MC3T3-E1 cells. Additionally, BBGs in the

composite cement stimulate new bone formation around the interface between the composite cement and the host bone at 8 and 12 weeks post-implantation. In contrast, PMMA bone cement alone primarily promotes the development of an intervening connective tissue layer, highlighting the superior bone-healing properties of BBG composites [50].

3.3. Cell adhesion

Cell adhesion, cell spreading, cytoskeletal organization, and the formation of focal adhesions are the four main sequential steps in the cell adhesion process [51]. The initial interaction between cells and biomaterials, which is crucial for cell differentiation and long-term stability, is mediated by ionic bonding or van der Waals forces.

This adhesion facilitates the interaction between the ECM and integrins or transmembrane receptors, leading to the formation of focal adhesion plaques. These adhesions occur when nanoclusters of activated integrins bind to the ECM and connect with cytoplasmic adaptor proteins (such as talins, vinculin, and paxillin), linking integrins to the actin cytoskeleton [52]. The ECM–integrin–cytoskeleton axis plays a key role in regulating cell-biomaterial adhesion and enables the transmission of chemical and mechanical signals into and out of the cell. Integrins serve as a bridge between the ECM and the cytoskeleton through focal adhesion components, activating focal adhesion kinase (FAK) and Src kinase. FAK phosphorylates paxillin and Crk-associated substrate (p130cas), allowing the signal adaptor protein to bond with the focal adhesion. This process is accompanied by actin assembly and dynamic changes in FAK, which are essential for cell adhesion, spreading, invasion, proliferation, and apoptosis [53](Fig. 3 Cell adhesion).

The study by Shie and Ding demonstrated that increasing the silicon content in cement resulted in higher total DNA content, pFAK, and total integrin levels. Cement with a higher Si/Ca molar ratio facilitated the expression of type I collage(COL I) and the α2β1 subintegrin, while calcium-rich cement promoted the expression of fibronectin (FN) and the αvβ3 subintegrin. Silicon was found to stimulate cell adhesion more effectively than calcium through the activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and p38 signaling pathways [54]. Additionally, it has been reported that mesoporous bioactive glass (MBG) extracts doped with vanadium can significantly upregulate the gene and protein expressions of Itga 2b (a member of the integrin alpha chain family), FAK, and phosphorylated ERK1/2 (p-ERK1/2). This upregulation promotes the proliferation and osteogenic differentiation of bone marrow stem cells (BMSCs), enhancing the expression of BMP2, COL I, and alkaline phosphatase (ALP) activity through activation of the Itga 2b–FAK–MAPK (pERK1/2) signaling pathway [55]. BBGs/BSBGs not only release silicon and calcium but also release boron, which can influence the expression of subintegrins. The enzyme linked immunosorbent assay (ELISA) results from Durand et al. showed that after treatment with the ionic dissolution products from boron-doped 45S5 BGs, integrin αvβ3 expression in the chorioallantoic membrane (CAM) was 2.5–3 times higher than in samples treated with Hanks' balanced salt solution. Additionally, Western blot analysis revealed greater expression of the β3 subunit of integrin αvβ3 in the boron-doped 45S5 BGs [56]. All of these findings indicate that BBGs/BSBGs can regulate cell adhesion through integrin signaling.

3.4. Alkaline microenvironment

When BBGs/BSBGs degrade, they release a substantial amount of ions or functional groups, leading to an increase in pH and the creation of a localized alkaline environment. This alkaline microenvironment at the interface between the materials and tissues may be a key factor contributing to the excellent bone repair performance of BBGs/BSBGs.

The acid-alkali balance plays a crucial role in bone remodeling and significantly impacts the activity of bone-related cells. The pH of the microenvironment can influence bone homeostasis by affecting the activity of both osteoblasts and osteoclasts (Fig. 3 Alkaline microenvironment). Acidic conditions, such as acidosis, can lead to bone loss. Initially, this was thought to be primarily due to the physicochemical dissolution of bone minerals. However, later research by Arnett et al. [57] revealed that acidic conditions activate osteoclasts, resulting in bone resorption. The acidification of the microenvironment significantly upregulates the expression of key enzymes in osteoclasts, including carbonic anhydrase II, vacuolar-type H^+-ATPase, cathepsin K, and tartrate-resistant acid phosphatase (TRAP).

The bone injury area may initially exist in an acidic microenvironment with a low pH [57,58]. This is often due to an interruption in local blood supply or an inability to establish compensatory blood flow around the bone wound, resulting in an ischemic and hypoxic environment. Under these conditions, anaerobic glycolysis is enhanced, leading to lactate accumulation. In addition, inflammation recruits inflammatory cells to the injury site, further decreasing the pH of the local microenvironment. Osteoblasts are highly sensitive to changes in H^+ concentration. Frick et al. [59] were the first to propose the effects of an acidic microenvironment on osteoblasts, suggesting that low pH inhibits ECM expression. Compared to the normal pH of 7.4, the ALP activity, cell mineralization, and collagen synthesis of osteoblasts are significantly reduced in an acidic environment [59]. Kato et al. [60] also outlined that acidic conditions can inhibit the differentiation and growth of osteoblasts. Subrahmanyam et al. [61] pointed out that acidic microenvironments can lead to imbalances in bone metabolism and contribute to bone-related diseases. It has been observed that the interstitial fluid around fracture sites tends to be acidic [58], and prolonged exposure to an acidic microenvironment can cause apoptosis of bone cells and osteoblasts, ultimately leading to bone loss [62]. Collectively, these studies demonstrate that under acidic conditions, osteoblast activity is suppressed, resulting in reduced new bone formation, while osteoclast activity is enhanced, increasing bone resorption and contributing to overall bone loss.

An alkaline environment is conducive to the differentiation of BMSCs into osteoblasts and can significantly inhibit osteoclast activity, thereby creating a more favorable osteogenic microenvironment. ALP activity is highly influenced by the pH of the microenvironment. As the pH increases within the range of 7.4–8.5, ALP activity is enhanced, whereas at a pH of 6.9, its activity drops to less than 10 % of its level under physiological conditions [63]. Therefore, under certain alkaline conditions, the high activity of ALP may support calcium deposition and the formation of calcium nodules, promoting bone formation. Our research has shown that as BSBGs degrade, the pH reaches above 8 after 24 h, which significantly enhances the proliferation of osteoblasts and the activity of ALP [64]. Conversely, when the pH is adjusted to neutral, osteoblast activity decreases. Alkaline conditions are known to promote the mineralization as demonstrated by Kimura et al. [65], who found that ambient alkaline environments support both cell proliferation and mineralization. These findings suggest that biomaterials can be designed to create an optimal alkaline environment, with a pH higher than the physiological norm (7.4) [64], to enhance bone regeneration. BGs have the ability to generate an alkaline material microenvironment, which may be one of the key factors in regulating osteogenic activity.

Studies provide evidence that during the degradation of BBGs/BSBGs, the release of functional ions can create a localized alkaline microenvironment. Our research team reported that the number of $TRAP^+$ osteoclastlike cells and the expression of Cathepthin K in the BSBG group with a higher μe-pH (18B2P) was decreased when compared with those BSBGs in 6B2P and 18B4P(Fig. 4A, B and C). The surface pH of BSBGs can reach 8–8.5 and be maintained for up to 150 h (Fig. 4D and E) [66]. As the alkalinity increases gradually, the fusion area of osteoclasts progressively decreases. At a pH of around 8.0, TRAP-positive osteoclasts were no longer generated. When the pH reached 8.5, boron significantly enhanced the activity of osteoblasts [64]. The spatiotemporal distribution of H^+ ions at the interface of BSBGs was monitored (Fig. 4), revealing that the distribution of H^+ on the BSBGs surface was

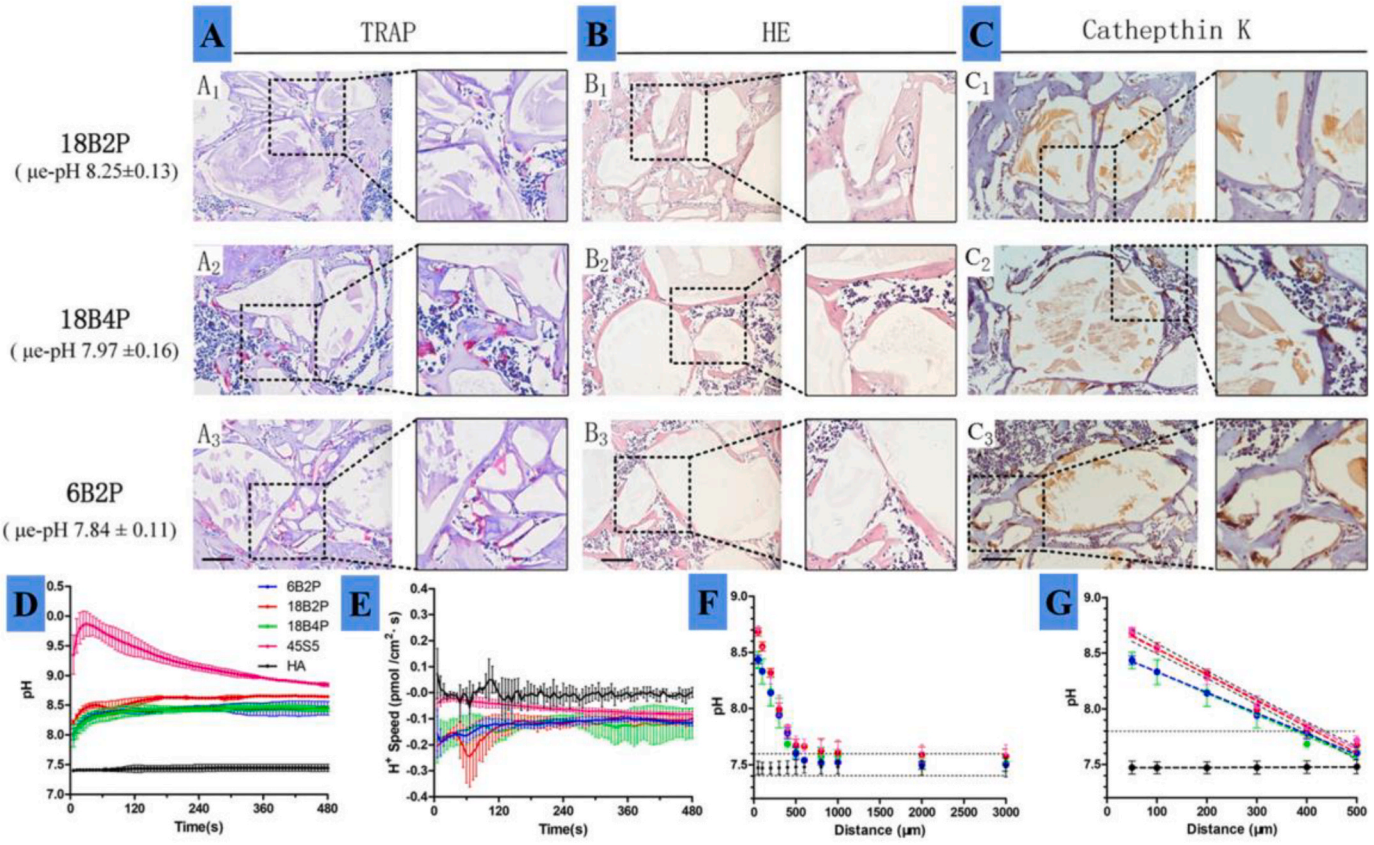

Fig. 4. Microenvironment boundaries by correlating spatiotemporal H^+ distribution with osteoclast behaviors
TRAP(A), HE(B) and Cathepthin K IHC(C) staining for mouse bone defects filled with BSBGs (6B2P, 18B2P, or 18B4P) at week 2; Time-dependent pH variation(D) and H^+ flux(E) at 50 μm away from the surface of BSBGs; Spatial H^+ distribution(G,F) near the surface of examined BSBGs [66].

uniform and closely associated with the "switch on/off" of osteoclast activity. By correlating the interfacial H^+ distribution with the "on/off" behavior of osteoclasts, our team found that the microenvironmental boundary of the material was approximately 400 ± 50 μm(Fig. 4F and G), which is broader than the generally accepted value. In osteoporotic mice implanted with materials exhibiting higher interfacial pH values and wider effective ranges, osteoclast activity was significantly reduced, and new bone was thicker. These findings suggest that a mildly alkaline microenvironment promotes bone regeneration by suppressing abnormally activated osteoclasts [66].

Our results also show that the alkaline pH generated by the degradation of BSBGs enhances the activity of strontium and boron, and facilitates the nucleation of apatite [64]. The alkaline microenvironment created by BSBGs promotes osteoblast proliferation and differentiation, increases ALP expression, and upregulates the expression of osteogenic-related genes, further supporting new bone formation [67]. Molecular mechanism studies indicate that BSBGs can upregulate the BMP/Smads pathway, enhancing new bone formation and demonstrating superior osteogenic performance [68,69].

Therefore, the alkaline environment produced by the degradation of BBGs/BSBGs effectively promotes bone repair. Endowing implant biomaterials with an alkaline material microenvironment may be an effective strategy for enhancing bone regeneration. As biomaterials degrade, a localized alkaline environment forms, influencing the activity of both osteoblasts and osteoclasts to improve and promote bone regeneration. Controlling the pH at the material-tissue interface is one of the key factors for optimizing the osteogenic properties of biomaterials.

3.5. Signal pathways related to osteogenesis induced by the ion microenvironment of BBGs/BSBGs

As research on BGs continues to evolve, the concept of "bioactivity" has continued and extended beyond the material's ability to induce hydroxyapatite formation and establish chemical bonds with tissues. The interactions between the material and cells, as well as the cellular responses elicited, are now recognized as key criteria for evaluating the biological activity of BGs [2,5]. BBGs/BSBGs stimulate bone remodeling not only by promoting hydroxyapatite formation but also by inducing bone regeneration through the ion microenvironment they create.

BBGs/BSBGs exhibit excellent chemical activity and a high coefficient of thermal expansion. Compared to SBGs, BBGs/BSBGs degrade faster and more completely. After implantation, they gradually degrade, releasing ions that form an ion-rich microenvironment. In addition to silicon, calcium, and phosphorus ions, BBGs/BSBGs release boron and other functional ions doped such as sodium, manganese, strontium, copper, zinc, silver, iron, and cobalt [30,33]. The ion microenvironment formed by these elements imparts specific functions to BBGs/BSBGs, which can directly or indirectly mediate cellular responses. The controllable degradation of the materials, release of ions, and interaction with surrounding cells, resulting in the unique VitaFlux adaptive regenerative characteristics of BBGs/BSBGs.

There are numerous reports the effects of BBGs/BSBGs on osteogenic processes, including osteoblast proliferation, differentiation, functional expression, and bone formation. However, the detailed molecular mechanisms involved are still in the exploratory phase. The signal pathways related to the osteogenic effects of BBGs/BSBGs (Fig. 5) are discussed below.

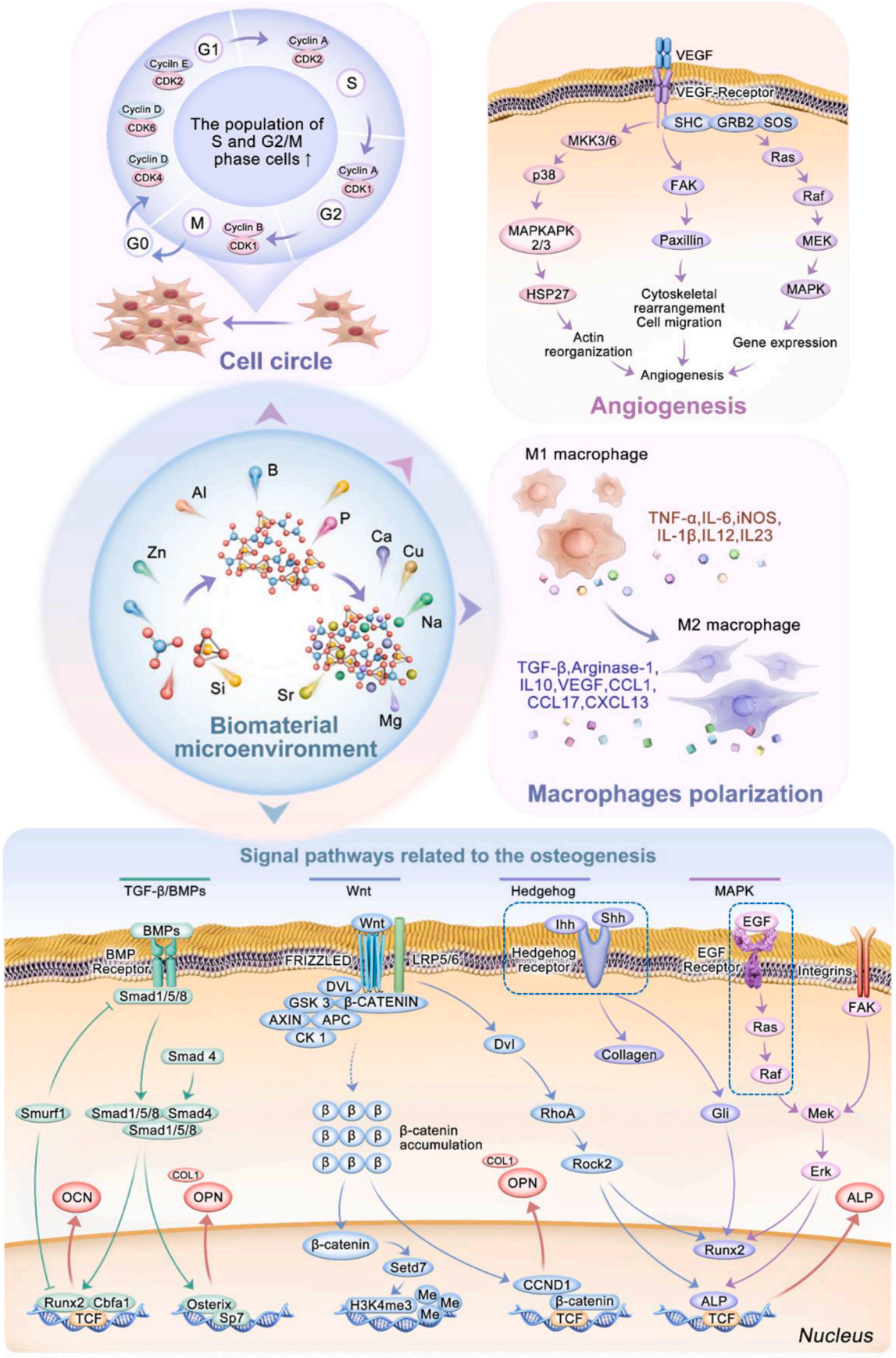

Fig. 5. Osteogenesis mechanisms of borate/borosilicate bioactive glasses.

3.5.1. Cell proliferation and cell cycle

Numerous studies have investigated the effects of BGs on the proliferation of mesenchymal stem cells (MSCs), osteoblasts, and other osteoblast-related cells. BGs have been shown to enhance cell adhesion, spreading, proliferation, and the expression of specific osteoblastic markers [70,71]. Research by Hench, Xynos, and Polak indicated that the mechanism behind in situ tissue regeneration involves the upregulation of seven gene families that control the osteoblast cell cycle, mitosis, and differentiation [13].

Cell cycle regulators, such as G1/S-specific cyclin D1, cyclin-dependent kinase inhibitor 1, cyclin I, and cyclin K, were activated when human osteoblasts were incubated with ionic products derived from BGs [72,73]. Additionally, there was an increase in the cell populations in both the S phase (DNA synthesis) and G2/M phase (mitosis) of the cell cycle when osteoblasts were cultured with BGs [74]. Osteoblasts exposed to BGs exhibited vacuole formation, with the vacuoles containing 75 % more silicon than other regions of the cell [75].This suggests that silicon released from BGs may play a role in the enhanced cell viability observed in BG-treated cells.

The incorporation of dopants such as boron in bioactive glasses enhances bioactivity and promotes cell proliferation by influencing the ECM and the release of ions like calcium, boron, and silicon. These ions are critical for cell cycle regulation (Fig. 5 Cell cycle) and the activation of osteogenic markers. Studies have demonstrated that incorporating boron into MBG scaffolds significantly improved osteoblast proliferation [76]. BBGs have been shown to enhance proliferation, osteogenic differentiation, and the expression of angiogenic factors in rat BMSCs [77]. Gorin et al. demonstrated that BBG scaffolds supported cell proliferation, bone regeneration, and osteoclast/osteoblast activity in vivo [78]. A significant increase in human adipose stem cell (ADSC) populations was observed both on and inside SBG (1393) scaffolds and around BBG (1393B20) scaffolds. Additionally, at two months, there was a marked increase in bone volume/tissue volume (BV/TV) values, with significantly higher filling volumes in the 1393B20 scaffolds compared to the 1393 scaffolds. Ion-doped BBGs/BSBGs also promote cell proliferation and viability [79]. Our group demonstrated that on the surfaces of Sr-doped BSBGs/PMMA composite cements, MC3T3-E1 cells exhibited discernible filopodia, and the material extracts showed a significantly higher relative cell growth rate [50]. Further research suggests that Sr^{2+} increases MSC proliferation by increasing the population of cells in the S and G2/M phases of the cell cycle, thereby contributing to enhanced osteogenic viability [80].

However, it was also noted that a higher B_2O_3 content in the glass, while increasing the conversion rate to hydroxyapatite (HA), resulted in greater inhibition of cell proliferation under static culture conditions [30]. This inhibition could be alleviated by using glasses with lower B_2O_3 content, by culturing cells under dynamic rather than static conditions, or by partially converting the glass to HA before cell culture [81].

3.5.2. Wnt/β-catenin signaling pathway

Numerous studies have demonstrated that BBGs/BSBGs significantly upregulate the expression and secretion of various markers associated with osteogenic differentiation and mineralization in cells, such as ALP, collagen type I (Col I), mineral production, osteocalcin (OCN), osteopontin (OPN), transcription factors RUNX2, Osterix (OSX), DLX5, and numerous nuclear co-regulators [82]. Further mechanistic investigations have revealed that these effects are linked to several signaling pathways involved in osteogenesis.

Biomaterial microenvironment of BBGs/BSBGs regulate osteogenesis through cell proliferation, related signaling pathways (BMP, Wnt, Hedgehog and MAPK), macrophage polarization and angiogenesis.

The canonical Wnt signaling pathway plays a critical role in regulating bone homeostasis. Activation of this pathway has been shown to increase bone mass and strength, while its inhibition leads to a decrease in these parameters [83]. Research from our team has indicated that strontium (Sr)-incorporated BBG cement enhances osteogenic potential by activating the Wnt/β-catenin signaling pathway in human BMSCs [84]. Another investigation revealed that Sr^{2+} activates non-canonical Wnt signaling, which regulates the expression and distribution of the Par complex, thereby influencing cell division [80].

Yin et al. [85] demonstrated that the Wnt/β-catenin signaling pathway is activated in both MBG and boron-containing MBG (B-MBG). The B-MBG group showed higher levels of β-catenin, phosphorylated glycogen synthase kinase-3β (p-GSK-3β), and the target protein Setd7. Setd7, a type of protein lysine methyltransferase, is known to methylate both lysine 4 of histone 3 (H3K4) and lysines on over 30 non-histone proteins. These results suggest that the Wnt/β-catenin signaling pathway is particularly activated in new bone areas associated with BBGs. Furthermore, increased localization of Setd7 and H3K4me3 was observed in Runx2-positive cells in defects treated with B-MBG scaffolds. When Setd7 was knocked down, the osteoblast differentiation of hBMSCs was inhibited, and this inhibition could not be rescued by extracts from B-MBG scaffolds. This confirms the critical role of Wnt downstream protein Setd7 in osteoblast differentiation and bone regeneration (Fig. 5 Signaling pathways related to the osteogenesis).

Another study by the same team reported that transcription factor 7-like 2 (TCF7L2), a member of the TCF/LEF domain family and a downstream effector of β-catenin in the Wnt/β-catenin signaling pathway, serves as a key promoter of B-MBG-induced bone regeneration and osteogenesis through lipocalin 2 (LCN2) [86]. TCF7L2 was highly expressed in osteoblasts treated with B-MBG scaffold extracts compared to MBG. LCN2, a secreted bone factor, positively influenced the osteogenic differentiation of MC3T3-E1 cells and promoted osteogenesis in vivo, which was induced by TCF7L2. When TCF7L2 was blocked, the osteogenic differentiation of osteoblasts was reduced. All the above highlights the importance of the Wnt/β-catenin signaling pathway in the osteogenic effects of BBGs/BSBGs, contributing to their potential in bone tissue engineering applications.

3.5.3. MAPK signaling pathway

MAPKs play a crucial role in regulating the key transcriptional mediators of osteoblast differentiation. Specifically, ERK and p38 MAPKs phosphorylate the C-terminal P/S/T domain of Runx2, the master regulator of osteoblast differentiation [87]. Hench, Xynos, and Polak were the first to propose that BGs can regulate the expression of osteogenic genes and control cellular responses at the genetic level [13]. Subsequently, Christodoulou, Hench, Polak et al. reported a 24-h treatment result with the ionic dissolution products of sol–gel 58S BGs indicating that the MAPK signaling pathway was stimulated in the cells [88]. Studies have shown that BG-incorporated composite scaffolds can significantly promote the proliferation, mineralization, and differentiation of BMSCs into osteoblasts by activating the Erk1/2-Runx2 pathway [89]. Au et al. proposed that BGs might transmit signals and stimulate MAPK pathways through integrin receptors. Once phosphorylated, MAPKs can translocate to the nucleus to activate target genes, including the *c-Jun* immediate-early gene, which supports the integrin-mediated intracellular signaling pathway: Integrins→FAK→ MAPK signaling cascade →AP-1 transcription factor complex (c-Jun) → Proliferation → Differentiation [90]. As mentioned in Chapter 3.3 on cell adhesion, BBGs/BSBGs, due to their silicon or calcium content, may regulate the MAPK signaling pathway via integrin activation. However, it remains unclear whether boron also plays a role in this signaling process. Besides integrins, it is unclear whether BG can act on MAPK through other upstream signaling pathways such as epidermal growth factor (EGF). Further research is needed to clarify boron's involvement in the upstream MAPK pathway (Fig. 5 Signaling pathways related to the osteogenesis).

3.5.4. BMP signaling pathway

BMPs are a group of growth factors that belong to the TGF-β superfamily and are key regulators involved in regulating biological processes

such as cell proliferation, differentiation, and bone morphogenesis [91]. The BMP signaling pathway is considered one of the key regulators associated with the osteogenesis mechanism of BBGs/BSBGs. BSBGs have been shown to promote the expression of the BMP2 gene during the early, middle, and late stages of osteogenic differentiation in a manner dependent on BSBG content [77]. Another study reported that critical genes in the BMP pathway, such as Smad1/5 and Dlx5, as well as p-Smad1/5 proteins, are significantly upregulated following BBG transplantation. These effects could be blocked by a BMP/Smad-specific inhibitor, indicating the pathway's central role. These findings suggest that BBGs induce osteogenic differentiation in BMSCs by activating the BMP/Smad pathway, which in turn promote the repair of large segmental bone defects [69]. This pathway plays a pivotal role in facilitating bone regeneration when using BBGs and BSBGs(Fig. 5 Signaling pathways related to the osteogenesis).

3.5.5. Hedgehog signaling pathway

The Hedgehog (Hh) signaling pathway is evolutionarily conserved and plays an essential role in various developmental processes, including embryonic tissue patterning and postembryonic tissue regeneration [92]. The Hh signaling pathway consists of the ligand and cell surface molecules, Patched (Ptch) and Smoothened (Smo). Activation of the Smo protein leads to the activation of the transcription factor Gli1, which then translocates to the nucleus, regulates the transcription of various genes, and then influences cell growth, proliferation, and differentiation [93]. The role of the Hh signaling pathway in bone development is well established [92]. It directs the differentiation of BMSCs toward osteoblasts and directly or indirectly affects osteoblast proliferation, differentiation, maturation, and overall bone formation. There is increasing evidence suggesting that the Hh signaling pathway is involved in bone formation and is influenced by bioactive materials, potentially contributing to the osteogenic effects of BGs [93].

An interesting finding is the up-regulation of Indian hedgehog (Ihh) between days 9 and 12 in 45S5 BG cultures [94]. Ihh, a member of the Hedgehog family of secreted ligands, is recognized as a master regulator of bone development, coordinating chondrocyte proliferation and differentiation. Based on their experimental data, the authors propose the hypothesis that BGs may also accelerate endochondral bone repair in vivo if they can enhance the biomineralization of the cartilaginous matrix in vitro. Zhang et al. provided further in vitro evidence that BG ceramics can influence the Hh pathway [95]. Real-time RT-PCR and Western blotting assays revealed that the expression levels of osteoblast-related genes (BMP2, OCN, ALP, Runx2) and Hh signaling pathway molecules (Gli1, Smo) were significantly higher in the bioactive glass coating group compared to the control group. Furthermore, when treated with the Smo inhibitor cyclopamine, the expression of Smo and Gli1 in BMSCs was dramatically downregulated in the bioactive glass coating group compared to the control group. Both mRNA and protein expression levels of osteogenesis-related factors were also downregulated in BGs coating groups after treatment with the Smo inhibitor. However, these results have yet to be tested in vivo, suggesting the need for further studies to confirm the role of BGs in modulating the Hh signaling pathway during bone repair and regeneration.

There is few direct research on the modulation of the Hh signaling pathway specifically by BBGs/BSBGs. However, the Hh pathway is crucial in bone repair, influencing osteoblast differentiation and bone homeostasis. Ions released by BBGs/BSBGs can modulate cellular signaling pathways, including those linked to osteogenesis like Wnt and BMP, and may indirectly influence Hedgehog signaling due to their effects on cellular proliferation and differentiation (Fig. 5 Signaling pathways related to the osteogenesis).

3.5.6. Other signaling pathways

In addition to the pathways discussed earlier, several other signaling pathways are involved in the osteogenesis facilitated by BBGs, either directly or indirectly. These include the RANK-RANKL, PI3K/AKT, and VEGF pathways, among others. The receptor activator of nuclear factor-κB(RANK)- RANK ligand (RANKL)signaling pathway, a classic regulator of bone metabolism, plays an important role in osteoclastogenesis. When RANKL binds to the RANK receptor on the surface of cells, it activates a series of downstream signaling pathways, including nuclear factor-κB (NF-κB), p38, c-Jun N-terminal kinase (JNK), ERK, and Src pathways. Huang et al. demonstrated that strontium-substituted BGs (Sr-SBG) significantly inhibited RANKL-mediated osteoclastogenesis, as evidenced by decreased formation of mature osteoclasts and down-regulation of osteoclastogenesis-related gene expression [96]. Furthermore, Sr-SBG was found to suppress osteoclastogenesis through the combined effects of strontium and silicon, which inhibited RANKL-induced activation of the p38 and NF-κB pathways. Additionally, research suggests that lithium-containing silicate bioceramics may also suppress RANKL-induced osteoclastogenesis in macrophages in vitro, further inhibiting osteolysis in vivo [97]. Our group's in vitro studies also indicated that the initial increase in pH due to the release of alkaline ions from the BSBGs significantly inhibited the activity of osteoclasts. The expression of RANK-RNAKL downstream osteoclastogenesis-related genes including nuclear factor of activated T-cells (NFTAc1), TRAP, matrix metalloproteinase 9 (MMP9), and Cathepsin K, were significantly reduced in RANKL-induced RAW 264.7 cells after culturing on the BSBGs/PMMA cement [98]. These findings underscore the importance of RANK-RANKL in regulating bone resorption, as well as the potential of BBGs/BSBGs to modulate these pathways for enhanced bone regeneration and repair. In the following sections, we will elaborate further on the roles of PI3K/AKT and VEGF in this process. There are few reports on the relationship between other signaling pathways closely related to osteogenesis, such as Notch, and BBGs/BSBGs, and further research is needed to determine their specific roles.

3.5.7. Cross-talk between different signaling pathways

We have highlighted the significant contributions of various aspects of the osteogenesis process in BBGs/BSBGs. Furthermore, the intricate crosstalk between signaling pathways such as integrin, Wnt, BMP, MAPK, Hedgehog, NF-κB, PI3K/AKT pathways and others is underlying causes to the gene regulatory networks that orchestrate osteoblast differentiation and bone development [99].

BGs create alkaline and ionic microenvironments, and the incorporation of different doped ions maybe can lead to synergistic effects between ions, thereby influencing multiple signaling pathways, which may potentially form cross-talk interactions between them. Our previous results showed that in the BG group, only Wnt/β-catenin signaling was upregulated, while the $SrCl_2$ group only activated the NFATc signaling pathway. However, mRNA and protein expressions related to NFATc and Wnt/β-catenin signaling pathways were both upregulated in response to strontium-substituted sub-micron BG (Sr-SBG). This suggests that strontium activated the NFATc pathway, while silicate activated the Wnt/β-catenin pathway, both working synergistically to enhance osteogenesis in Sr-SBGs [36].

There is also a complex interaction between integrin and MAPK signaling pathways. Regarding cell attachment, cells cultured on SBGs (S53P4) and BSBGs (1–06) showed enhanced production of integrin β1 and vinculin. Focal adhesions on these materials were smaller but more dispersed compared to those on standard polystyrene culture plastic. FAK, ERK1/2, and JNK-induced c-Jun phosphorylations were all upregulated upon BG contact. When cells were incubated with FAK and MAPK inhibitors, BSBG-induced osteoinduction was significantly reduced, highlighting the important role of FAK and MAPKs signalings in BSBG-induced early osteogenic commitment of human adipose stem cells (hASCs). These findings demonstrate the role of direct cell-BSBG interactions and offer valuable insights into the integrin and MAPK synergistic effects of multiple signaling pathway mechanisms in BG-induced osteogenic differentiation [100].

The interaction between Wnt/β-catenin and MAPK signaling is

particularly important in regulating osteoblast activity. Studies have shown that borosilicate glasses can influence the MAPK pathway, which subsequently boosts Wnt signaling, enhancing the activation of proteins like LRP6, which is essential for bone growth and matrix mineralization. This cross-talk between Wnt and MAPK plays a critical role in the proliferation of osteoblast-related cells [33,101]. Meanwhile, BMP/Smad and Wnt/β-Catenin pathways has synergistic interactions. BMP signaling boosts Wnt pathway activity by increasing β-catenin transcription. This interaction promotes osteogenic marker expression and bone matrix deposition [102]. Boron-doped glasses have been observed to activate both BMP and Wnt pathways, which collectively contribute to bone morphogenesis and cellular differentiation. This interaction enhances bone repair, making these glasses highly beneficial in tissue engineering applications.

Along with BMP and Wnt, pathways like Hh are influenced by ion release from BBGs/BSBGs, contributing to angiogenesis and osteogenesis. For example, ion release, including boron, impacts the expression of genes related to these signaling pathways, promoting better integration between cells and the surrounding matrix [33,101].

On the other hand, some pathways exhibit antagonistic interactions during osteogenesis. For example, inflammation related signaling pathway, NF-κB, suppresses β-catenin activity to decrease osteoblast differentiation [103]. Several studies emphasized the fracture risk rise in inflammatory bone loss, and inhibiting inflammation could alleviate reduced bone quantity and quality [104]. Controlling inflammation via ion-releasing BBGs/BSBGs that modulate macrophage polarization toward the M2 phenotype may mitigate this antagonism. The following Chapter 3.5 will provide a detailed introduction to BBGs/BSBGs and macrophage polarization.

These results underscore the ability of BBGs/BSBGs to influence the complex interplay among signaling pathways in their induced osteogenesis, emphasizing the importance of the synergistic effects between various cellular and molecular mechanisms. Although the precise mechanisms remain under investigation, current findings reveal significant cross-talk between different pathways. These interactions, whether synergistic or antagonistic, are pivotal in determining bone regeneration outcomes and may guide the design of BBGs/BSBGs with enhanced therapeutic efficacy. Strategically selecting doping ions and regulating their release can significantly enhance these synergistic pathways. For instance, strontium activates the PI3K/AKT pathway [105] and NFATc signaling pathway [36], and silicate activated Wnt/β-catenin signaling pathway [36], thereby maybe promoting synergy between Wnt and BMP signaling. Similarly, copper stimulates HIF-1α and drives angiogenesis [105–107] and may indirectly supporting the effects of PI3K/AKT and BMP/Smad pathways. Gradual ion release is crucial to ensure sustained activation of synergistic pathways without overwhelming cellular systems. While by improving anti-inflammatory properties and designing multifunctional BBGs/BSBGs scaffolds (for example, angiogenesis and osteogenesis integrated functionality and time-dependent modulation) with 3D-printed structures, these materials are supposed to mitigate antagonistic influences like NF-κB overactivation and may achieve superior therapeutic efficacy. These integrative approaches may offer a promising pathway for advancing the clinical translation of BBGs/BSBGs materials in regenerative medicine.

3.6. Macrophage polarization

The immune response triggered by bone implant materials can affects and determines the biological behavior of bone cells, which can lead to two very different outcomes. One possibility is that the process results in a foreign body response, where immune cells encapsulate the implant, permanently isolating it from the surrounding tissue, leading to implant failure. The other, more favorable outcome is when the immune response aligns with the natural bone healing process, promoting bone integration, angiogenesis, and the transport of oxygen and nutrients to the site of new tissue formation [108]. Among the cells involved in immune regulation, macrophages exhibit complex functionality and high plasticity during bone regeneration and remodeling [109]. Macrophages are key regulators of the bone immune system and have attracted significant attention because of their ability to either positively or negatively influence tissue remodeling after injury [110].

Mature macrophages undergo phenotypic and morphological changes in response to various factors, a process known as macrophage polarization [111]. According to their responses to different environmental stimuli, macrophages are primarily activated into two phenotypes: classically activated macrophages (M1) and alternatively activated macrophages (M2). Macrophages initially exist in an unpolarized state (M0), originating from monocytes. After bone injury, M0 macrophages rapidly infiltrate the injury site and transition into the pro-inflammatory M1 phenotype during the early stages of healing. M1 macrophages are critical in early repair as they perform debridement and promote early-stage angiogenesis and growth. As healing progresses, M1 macrophages shift to the anti-inflammatory M2 phenotype, guiding the completion of the repair process. Throughout osteogenesis, macrophages regulate the function of stem cells by releasing cytokines and chemokines, playing a crucial role in coordinating the phases of inflammation, repair, and regeneration [112].

Early polarization of macrophages to the M2 phenotype can result in the production of excessive fibrotic factors, leading to the formation of a fibrous capsule around the implant, which can cause implant failure. Conversely, if macrophages do not transition to the M2 phenotype at the appropriate time, the prolonged presence of M1 macrophages will lead to the release of excessive pro-inflammatory factors such as interleukin-1β(IL-1β), IL-6, and tumor necrosis factor-α(TNF-α). This can cause chronic inflammation, delayed healing at the defect site, and ultimately implant failure. Therefore, strictly controlling the timing of macrophage activation and the transition between M1 and M2 phenotypes is critical for the successful integration of biomaterials in transplantation.

Previous studies have demonstrated that different biomaterials can influence the phenotype and polarization of macrophages [112–114]. BGs exhibit good biocompatibility and generally do not trigger adverse immune reactions locally. Day and Boccaccini reported that 45S5 BGs significantly reduced the secretion of pro-inflammatory cytokines, such as TNF-α and IL-6, in stimulated cells compared to those stimulated without bioactive glass [114]. Further research on the effects of BGs on macrophage polarization revealed that BG particles support the appropriate development and function of macrophages without inducing polarization towards the pro-inflammatory M1 phenotype [115]. In fact, when incubated with strontium- or copper-containing BGs, macrophages were polarized towards the pro-regenerative M2 phenotype rather than the pro-inflammatory M1 phenotype [105,106,116]. It was found that strontium doped BGs may enhance mitochondrial function in macrophages by activating the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway, thereby promoting osteogenesis [105]. The PI3K/AKT/mTOR signaling pathway is a critical intracellular pathway that regulates various cellular processes, including cell survival, growth, proliferation, metabolism, and immune response. The pathway is typically activated when extracellular ligands bind to cell surface receptors, primarily receptor tyrosine kinases (RTKs) or G-protein-coupled receptors (GPCRs), leading to receptor dimerization and autophosphorylation, creating docking sites for phosphoinositide 3-kinase (PI3K) [117]. PI3K is then recruited to the plasma membrane and activated. Active PI3K phosphorylates the membrane lipid phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 serves as a docking site for proteins with pleckstrin homology domains, including AKT (also known as protein kinase B) and PDK1 (phosphoinositide-dependent kinase 1). PDK1 phosphorylates AKT at Thr308 and mTOR phosphorylates AKT for full activation [118]. Once fully activated, AKT dissociates from the membrane and phosphorylates numerous downstream targets involved in cell survival, growth, metabolism, and immune modulation. Strontium doped BGs

significantly enhance the mitochondrial function of macrophages, a critical aspect of cellular metabolism and energy production. Specifically, Sr^{2+} ions released from the BG matrix stimulate the phosphorylation of PI3K and its downstream effectors, AKT and mTOR, thereby increasing mitochondrial membrane potential and adenosine triphosphate (ATP) production within macrophages [105]. The improved mitochondrial function leads to enhanced cellular activity and a metabolic shift that supports macrophage polarization toward the anti-inflammatory M2 phenotype, creating a microenvironment conducive to osteogenesis. Additionally, this immunomodulatory effect promotes the secretion of anti-inflammatory cytokines, such as IL-10, while reducing the expression of pro-inflammatory mediators. As a result, Sr-BGs effectively remodel the immunological microenvironment at the injury site, providing a dual benefit of accelerating bone regeneration and mitigating chronic inflammation [105]. Further research is needed to determine which receptor upstream of PI3K biological glass specifically acts on. Not only strontium doped BGs, but copper doped glasses can also affect macrophage polarization. Incorporation of Cu^{2+} into BGs significantly promoted the proliferation and maturation of chondrocytes by activating the HIF pathway, which further shifted macrophages towards the anti-inflammatory M2 phenotype and increased the secretion of anti-inflammatory cytokines [106]. Copper ions enhance the stability and activity of hypoxia-inducible factor 1-alpha (HIF-1α), a transcription factor that responds to low oxygen conditions, and promote the expression of vascular endothelial growth factor (VEGF), which aids in angiogenesis and tissue repair [107]. Additionally, copper supports the secretion of anti-inflammatory cytokines, such as interleukin-10 (IL-10), facilitating the polarization of macrophages toward the M2 phenotype, and reduces the production of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-18 (IL-18), thereby suppressing M1 polarization [106].

Regarding BBGs/BSBGs, our team found that strontium- and copper-incorporated BBGs/BSBGs upregulated anti-inflammatory genes (interleukin-1 receptor antagonist and transforming growth factor–β1(TGF-β1) while downregulating pro-inflammatory genes (IL-1β and IL-6) in macrophages at 3 days [119]. Additionally, our team developed glucose and hydrogen peroxide dual-responsive BSBG scaffolds loaded with epigallocatechin gallate, designed to modulate the abnormal inflammation associated with diabetic alveolar bone defects. These scaffolds regulated macrophage polarization from the M1 to the M2 phenotype directly by promoting autophagy and lessening inhibition of autophagic flux, and indirectly by suppressing NF-κB activation in stem cells and restoring their immunoregulatory capacity [120]. The results showed that BSBGs containing boron and strontium (1393B2Sr8 BG) released B and Sr ions, which synergistically enhanced vessel regeneration, modulated M2 macrophage polarization, and promoted new bone formation both in vitro and in vivo. Interestingly, the 1393B2Sr8 BG was also found to mobilize monocytes from the spleen to the defect sites, where they subsequently differentiated into M2 macrophages. In rats without a spleen, fewer M2 macrophages were observed surrounding skull defects, and bone healing was slower, indicating the beneficial effects of circulating monocytes and polarized macrophages provided by the spleen, on bone regeneration [121]. These findings highlight the potential of BBGs/BSBGs in regulating macrophage polarization, making them promising candidates for accelerating bone healing through improved immunomodulation (Fig. 5 Macrophage polarization).

3.7. Angiogenic effects

Angiogenesis is a crucial aspect of bone reconstruction (Fig. 5 Angiogenesis), as promoting vascularization can significantly accelerate bone repair. Numerous studies have explored methods to enhance the angiogenic potential of BGs, and substantial progress has been made in the field of bone defect repair in recent years.

BBGs/BSBGs have demonstrated the ability to not only enhance proliferation and osteogenesis but also promote the expression of angiogenic factors in BMSCs. Notably, BSBGs have been shown to support the angiogenic differentiation of human umbilical vein endothelial cells (HUVECs) [77]. While BSBGs led to only a slight stimulation of osteogenesis and angiogenesis in vitro, they significantly enhanced vascularization in ovo, particularly at higher concentrations [122].

Durand et al. evaluated the pro-angiogenic capacity of boron-doped 45S5 BGs using the vasculature of the embryonic quail chorioallantoic membrane (CAM) model. Their experimental results demonstrated that the ionic dissolution products (IDP) released by boron-doped 45S5 BGs can stimulate blood vessel formation in the CAM system, which the authors attributed to the boron present in the IDP [56]. In a subsequent experiment, the research group cultured HUVECs in the IDP from boron-doped 45S5 BG scaffolds to further investigate the effects. The results showed that the IDP of the scaffolds stimulated HUVEC proliferation and migration, which were associated with the phosphorylation of ERK1/2, FAK, and p38 protein. Additionally, boron-doped 45S5 BGs enhanced HUVEC tubule formation in vitro and promoted the secretion of IL-6 and basic fibroblast growth factor (bFGF). These effects were attributed to the presence of boron in the ionic dissolution products [123].

In addition to high VEGF production, studies suggest that S53P4-based BSBGs stimulate the expression of endothelial markers such as von Willebrand Factor and platelet endothelial cell adhesion molecule-1 (PECAM-1) in human adipose stem cells (hASCs) [82]. Another mechanistic study revealed that the BSBGs (0106-B1-BG) groups exhibited higher mRNA and protein expression levels of VEGF, placental growth factor (PlGF), and endothelin-1 (EDN1) compared to the SBG groups.

PlGF, a member of the VEGF family, supports VEGF-driven endothelial cell proliferation and migration. EDN1 was identified as a key mediator in angiogenic signaling regulated by VEGF and TGF-β. The angiogenic effect of EDN1 is highly dependent on its cooperation with the VEGF system. The superior ability of 0106-B1-BG BSBGs to induce pro-angiogenic factors may be attributed to its influence on PlGF and EDN1, indicating an enhanced angiogenic potential of 0106-B1-BG BSBGs during bone regeneration [123–125].

These findings underscore the potent angiogenic and osteogenic effects of ion-doped BBGs/BSBGs in bone tissue engineering. BBGs/BSBGs may serve as an inexpensive inorganic angiogenic agent, offering a convenient alternative to the use of conventional angiogenic growth factors for enhancing vascularization in bone repair applications.

4. Disadvantages, strategies and prospects

BBGs/BSBGs are an innovative class of biomaterials with exceptional biological advantages and wide-ranging application prospects, and our team have carried out the first clinical investigation of BSBGs reinforced PMMA bone cement in a patient. After implantation for three months, apatite nucleation was found around the material by using the dual-energy CT (Fig. 6), indicating the biological interface was formed. This promising result highlights the potential application of this material in the future [98]. The study presents significant advancements in the clinical translation of BBGs/BSBGs. By combining bioactive BSBGs with PMMA bone cement, the researchers addressed several critical challenges in the clinical application of bioactive glasses. Their clinical significance lies in the ability to improve implant-bone integration while maintaining the mechanical properties of PMMA. The incorporation of bioactive strontium doping BSBGs not only enhances the osteoinductivity of PMMA, but also enables a more controlled and sustained release of therapeutic ions [98]. This controlled release is a key feature, as it modulates the biological environment to favor bone regeneration and reduce inflammation, thereby strengthening the interface between the implant and the host bone and improving patient outcomes. Additionally, the BSBGs provide an alkaline microenvironment. The increase in pH balances the activities of osteoclasts and osteoblasts. This creates an optimal physicochemical environment around the implant, supporting effective bone regeneration, ensuring long-term clinical success and

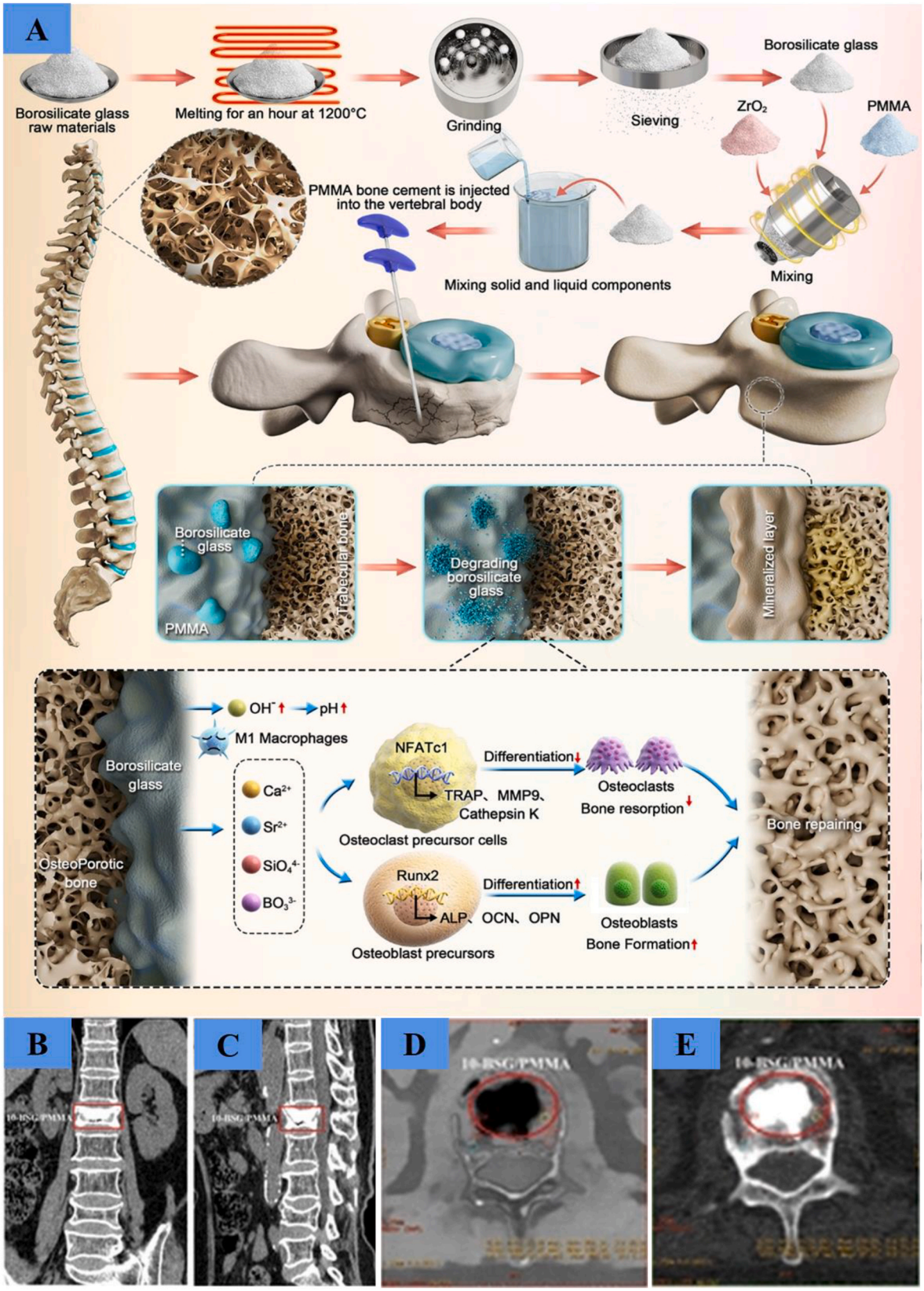

Fig. 6. The first clinical case of using BSBGs/PMMA cement to promote bone repairing [98,98].
Schematic diagram illustrating the preparation of BSBGs/PMMA cement (A); Analysis of L1 vertebrae after injection of BSG/PMMA cement to a 72 year-old female: CT plain scan (B, C), Dual-energy CT (DECT) water (HAp) base image (D)and HAp (water) base image (E)

bringing these materials closer to widespread therapeutic use. These findings pave the way for their translation from bench to bedside.

While BBGs/BSBGs offer promising therapeutic benefits, several challenges must be addressed to ensure their safe and effective clinical application. For instance, high concentrations of dopants, such as cerium and silver, can lead to cytotoxic effects, necessitating careful optimization of dopant levels to maintain bioactivity and biocompatibility [34]. Optimizing degradation rates is critical; excessive degradation not only leads to the rapid release of a large amount of ions, but also can compromise mechanical integrity, while insufficient degradation may hinder tissue regeneration [33]. The incorporation of dopants introduces complexities in regulatory approval processes, requiring extensive validation to meet safety and efficacy standards [126]. Additionally, scaling patient-specific designs through technologies like 3D printing, while maintaining affordability, remains challenging due to the need for precise control over material properties and manufacturing processes [126]. Furthermore, the long-term in vivo behavior of these materials, including their degradation products and interactions with host tissues, requires further study to ensure clinical reliability. Despite their superior mechanical strength compared to other bioactive glasses, BBGs/BSBGs still do not provide sufficient mechanical support in the early stages of healing when used on their own. As a result, most of them are currently limited to non- or low-load applications. To address these problems, researchers have proposed many methods to improve the mechanical properties [53]. One approach involves reinforcing BBGs/BSBGs by incorporating polymers (natural or synthetic) and fibers (carbon or ceramic fibers), or by doping ions into BBGs/BSBGs creates composite materials. For instance, a study demonstrated that the inclusion of BBGs in 3D-printed gelatin-alginate hydrogels significantly enhanced the physical properties and printability of the hydrogels [127]. Advanced manufacturing techniques such as 3D printing [128], surface coating and structural optimization may be employed to control porosity or obtain biomimetic designs, thereby enhancing mechanical properties while promoting cell attachment and proliferation [49], which are key areas of focus in the ongoing research on BBGs/BSBGs.

Additionally, further investigation is needed to better understand the relationship between the composition and structure of BBGs/BSBGs. The controllable degradation process of these materials is closely linked to their composition and structure. Establishing a comprehensive model that correlates composition, structure, and ion release will be crucial in guiding the future design of BBGs/BSBGs for specific biological applications. This model would provide valuable insights for optimizing both composition and structural design, ultimately enhancing the performance of these biomaterials in clinical applications.

The main distinction between BBGs/BSBGs and other bioactive glasses is that boron can also serve as a BG network-forming agent. Boron has multiple functions and is an essential element for human and animal health. It is closely linked to osteogenesis, as it is an important nutrient and plays a critical role in bone growth and metabolism. Appropriately increasing the concentrations of boron can upregulate the gene expression of collagen, OPN, OCN and bone sialoprotein (BSP), regulate bone growth hormone, and enhance calcium and bone metabolism. Conversely, a lack of boron can significantly inhibit osteogenic performance [129], slow growth, and even lead to conditions like osteoarthritis.

However, the bone regeneration mechanisms regulated by boron in BBGs/BSBGs still require further investigation. NaBC1, a Na^+-coupled borate transporter, is a borate-specific carrier channel present on the surface of mammalian cells that helps regulate internal boron homeostasis. In the absence of borate, NaBC1 mediates the transport of Na^+ and OH^-(H^+), but in the presence of borate, it functions as an electrogenic, voltage-regulated Na^+-coupled $B(OH)_4^-$ transporter. NaBC1 generates shallow inward rectification when mediating Na^+-$B(OH)_4^-$ influx and steep outward rectification during efflux [130]. At low concentrations (0.1–1 mM), borate can stimulate cell growth and proliferation by activating the classical MAPK signaling pathway through phosphorylation of MEK and ERK kinases. Despite its importance as a borate carrier, there are few studies investigating the regulatory relationship between BBGs/BSBGs and NaBC1. Therefore, whether the ion microenvironment formed by BBGs/BSBGs can exert osteogenic effects and maintain bone metabolism homeostasis through the borate-specific NaBC1 transporter remains unclear and requires further exploration. The specific role and mechanism of NaBC1 in this context need more in-depth study (Fig. 7).

Studies have shown that directly culturing cells on BBGs/BSBGs or in undiluted extract mediums can reduce cell proliferation [78], likely due to the cytotoxicity associated with excessive boron content [30,33]. The amount of boron in BBGs/BSBGs affects both the degradation rate and ion release. For example, in efforts to upregulate Wnt/β-catenin signaling, researchers incorporated lithium, a glycogen synthase kinase 3 antagonist, into BBGs and tuned them to release their constituent ions rapidly. However, while these materials were non-toxic at low concentrations in cell culture medium, their ability to effectively regulate Wnt/β-catenin signaling was limited at these lower concentrations of lithium release [131]. The increasing amounts of Si being released with higher boron concentrations may facilitate a collapse of the pore network and loss of internal surface area of sol-gel MBG. This loss in porosity may restrict the re-uptake of Ca in the form of HCA. Consequently, there is a compromise between Si release/pore collapse and Ca release/uptake [132].

Finding a balance between minimizing boron toxicity and maintaining its biological activity is crucial. There remains a gap in research on how to optimize boron content to balance material degradation and biological efficacy. To address this, scholars have suggested several potential solutions. These include using glasses with lower B_2O_3 content, culturing cells under dynamic conditions rather than static conditions, or partially mineralizing the glasses to HA before cell culture [81]. These strategies may help mitigate the inhibitory effects of boron on cell proliferation while preserving the material's beneficial properties.

As previously discussed, many aspects of cellular function are influenced by changes in the biomaterial microenvironment. However, the mechanisms behind the effects of pH changes in most of these areas, particularly in relation to cellular signal transduction associated with pH fluctuations caused by BBGs/BSBGs, are not well understood. Research on pH signal transduction related to BBGs/BSBGs is still limited. When the extracellular acid-base environment changes, cells are required to utilize their reserve of H^+ binding sites to regulate these fluctuations in pH. Among the key mechanisms involved are the transmembrane transport processes mediated by various ion channel proteins on the cell membrane, which help regulate cellular functions in response to pH changes. These processes require further exploration in the study of BGs, as they may hold critical insights into how BBGs/BSBGs influence cellular behavior through pH modulation.

For example, G protein-coupled receptors (GPCRs) play a crucial role in pH cascade reactions. GPCRs form the largest and most therapeutically targeted family of receptors on the surface of human cells. Unlike many cellular proteins, GPCRs are regularly exposed to a range of dynamic pH values, from pH 7.4 on the cell surface to pH 5.0 in the endosome. They can also be subjected to prolonged exposure to acidic microenvironments caused by conditions such as cancer and inflammation. Although the GPCR family contains over 800 members, only four types are known to be proton-sensitive GPCRs (pH GPCRs) and can be directly activated by pH [133]. These include ovarian cancer G protein-coupled receptor 1 (OGR1, also known as GPR68), T cell death-related gene 8 (TDAG8, also known as GPR65), GPR4, and G2A (also known as GPR132, though its function as an H^+ receptor remains controversial).

Low extracellular pH can cause protonation of different histidine residues on the extracellular surface of receptor cells, thereby activating pH GPCRs. RT-PCR experiments have confirmed the expression of GPR4, TDAG8, and OGR1 proton receptors in primary bone cells. Among these, OGR1, as an only G protein-coupled pH receptor, activates

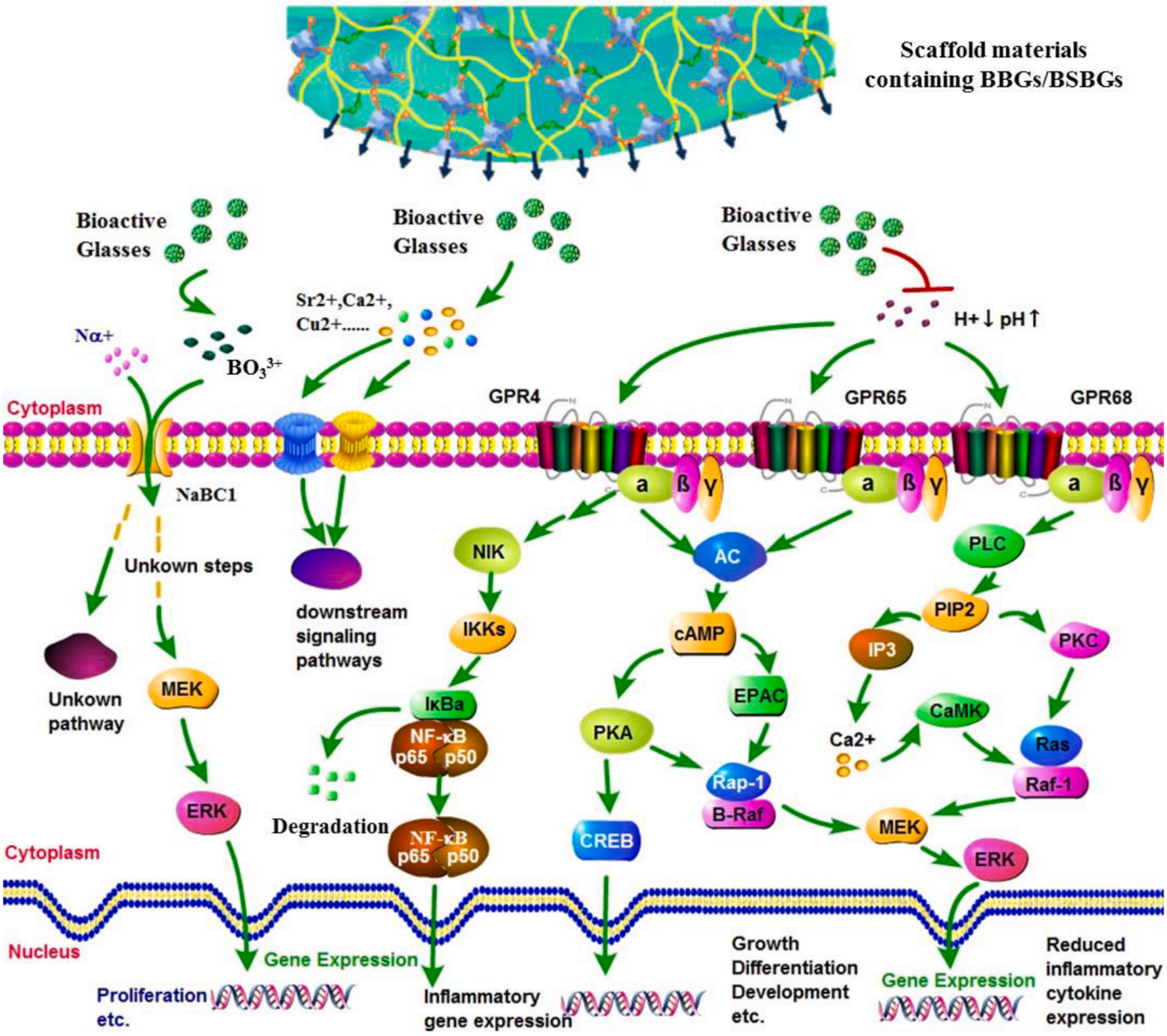

Fig. 7. Possible osteogenic mechanism of BBGs/BSBGs by pH GPCRs and NaBC1 signaling pathways.

phospholipase C, which in turn increases the metabolism of diacylglycerol and phosphoinositol (PI), leading to elevated intracellular calcium levels. Studies by Frick and Tomura et al. [134,135] suggest that OGR1 is a preferred candidate for an osteoblast proton sensor. Extracellular acidic pH activates OGR1, leading to an increase in intracellular calcium, which acts as a second messenger mediating the effects of acidosis on osteoblasts. This includes the upregulation of cyclooxygenase −2 and RANKL, which enhances bone resorption by osteoclasts. Moreover, as pH decreases, OGR1 expression in endothelial progenitor cells (EPCs) increases, and OGR1 can inhibit EPC proliferation and cause cell cycle arrest. TDAG8 has been shown to inhibit bone resorption caused by ovarian resection, while its deficiency promotes osteoclast formation and enhances calcium absorption by osteoclasts. Acidic environments can alter osteoclast morphology via the TDAG8/-cyclic adenosine monophosphate/Ras homologous (TDAG8/-cAMP/Rho) signaling pathway. DAG8 regulates the inhibitory effect of extracellular acidification on the secretion of pro-inflammatory cytokines in mouse macrophages. This has led scholars to propose that there may be a balance between OGR1 and TDAG8—two pH GPCRs—to maintain normal osteoclast formation and function. GPR4, on the other hand, can upregulate the expression of inflammatory mediators such as IL-1β, IL-6, and TNF-α by promoting intracellular cAMP in acidic environments. It also participates in various pathological processes, including cellular immunity, inflammation, and tumorigenesis. As proton receptors, pH GPCRs are closely related to the cellular acid-base microenvironment.

However, it remains unclear whether the alkaline microenvironment provided by bioactive glasses can influence the osteogenic differentiation mechanisms mediated by these pH GPCRs in specific microenvironments. Further research is needed to understand whether the alkaline conditions formed by BGs affect the function of these transporters, as any impact on their activity could significantly influence cellular function. BBGs/BSBGs are known to alter the cellular microenvironment, and if they modulate the function of these transporters, they could have profound effects on cellular behavior, which need to be gone deep into.

The mechanisms by which BBGs/BSBGs regulate bone tissue

regeneration still require further exploration. Currently, there is a lack of sufficient research on the effects of BBGs/BSBGs on bone tissue repair and their interactions with the surrounding microenvironment. The biological effects of BBGs/BSBGs in vivo are influenced by complex dynamic microenvironments and host reactions, involving interactions with multicellular systems. Therefore, in addition to their osteogenic effects, it is crucial to study the challenges they face at various levels throughout the life cycle of the organism. Particularly, more attention should be given to understanding the bone regeneration mechanisms driven by immune microenvironment regulation, the synergistic effects of BBGs/BSBGs with growth factors, and their roles in promoting angiogenesis and exhibiting anti-tumor effects. By addressing these key areas, a solid theoretical foundation and practical knowledge can be provided for the clinical application of BBGs/BSBGs, ultimately enhancing their therapeutic potential in bone tissue engineering and beyond.

5. Conclusion

BBGs/BSBGs play a crucial role in bone repair by promoting cell adhesion, proliferation, differentiation, and modulating cellular activity and function. These VitaFlux glasses actively respond to biological environments and release beneficial ions as continuous flow of life-giving energy, repairing, adjusting, and adapting to environmental changes. Over the past few decades, extensive research has been conducted to explore the molecular mechanisms underlying BBGs/BSBGs. This review highlights recent advances in understanding their osteogenesis mechanisms. Due to their ability to promote osteogenesis, angiogenesis, and favorable interactions with the immune system, BBGs/BSBGs present a unique and highly promising third-generation bone repair materials. Despite these advantages, challenges remain, particularly in understanding the exact mechanisms by which these materials regulate bone tissue repair, their effects on the surrounding microenvironment, and their interactions with signaling pathways such as Wnt/β-catenin, MAPK, BMP, and Hedgehog. Furthermore, we proposed the possible hypothesis role of NaBC1 and pH GPCRs, such as OGR1 and TDAG8, in BBGs/BSBGs osteogenesis regulation, which may significantly impact osteoclast and osteoblast function.

CRediT authorship contribution statement

Xian Li: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Kun Su:** Visualization, Writing – review & editing. **Limin Zhao:** Visualization. **Hao Zhang:** Visualization. **Qiang Yang:** Writing – review & editing. **Ping Du:** Writing – review & editing. **Xiaofeng Chen:** Writing – review & editing, Funding acquisition. **Haobo Pan:** Writing – review & editing, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

The current review does not involve any experimental work on human subjects or animals, and thus does not require approval from an ethics committee.

Declaration of competing interest

Prof. Haobo Pan is an editorial board member for Bioactive Materials and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2023YFC2416900), Shenzhen Medical Research Fund (B2302031), Guangdong Marine Economic Development Program (GDNRC[2023]35), National Natural Science Foundation of China (No. 32161160327 and U23A20692), Guangdong Basic and Applied Basic Research Foundation (2022B1515120048, 2023A1515011727), Shenzhen Science and Technology Program (JSGGKQTD20210831174330015 and JCYJ20220818101613028). Reproduced from Ref.12 with permission from The Royal Society of Chemistry, and Ref.48 and 80 with permission from the American Chemical Society.

Abbreviations

BBGs/BSBGs	borate and borosilicate bioactive glasses
BGs	bioactive glasses
HCA	hydroxycarbonate apatite
SBGs	silicate bioactive glasses
BBGs	borate bioactive glasses
BSBGs	borosilicate bioactive glasses
RANKL	receptor activator of nuclear factor-κB ligand
BMP	bone morphogenetic protein
HA	hydroxyapatite
SBF	simulated body fluid
ECM	extracellular matrix
PMMA	poly(methylmethacrylate)
FAK	focal adhesion kinase
FN	fibronectin
COL I	type I collage
MAPK	mitogen-activated protein kinase
ERK	extracellular signal-regulated kinase
MBG	mesoporous bioactive glass
p-ERK	phosphorylated extracellular signal-regulated kinase
BMSCs	bone marrow stem cells
ALP	alkaline phosphatase
ELISA	enzyme linked immunosorbent assay
CAM	chorioallantoic membrane
TRAP	tartrate resistant acid phosphatase
MSCs	mesenchymal stem cells
ADSC	adipose stem cell
OCN	osteocalcin
OPN	osteopontin
OSX	Osterix
GSK-3β	glycogen synthase kinase-3β
H3K4	lysine 4 of histone 3
TCF7L2	transcription factor 7-like 2
LCN2	lipocalin 2
EGF	epidermal growth factor
Hh	Hedgehog
Ptch	Patched
Smo	Smoothened
Ihh	Indian hedgehog
RANK	receptor activator of nuclear factor-κB
NF-κB	nuclear factor-κB
JNK	c-Jun N-terminal kinase
NFATc	nuclear factor of activated T-cells
MMP	matrix metalloproteinase
hASCs	human adipose stem cells
IL	interleukin
TNF-α	tumor necrosis factor-α
PI3K	phosphatidylinositol 3-kinase
AKT	protein kinase B
mTOR	mammalian target of rapamycin
RTKs	receptor tyrosine kinases
TGF-β1	transforming growth factor-β1
HUVECs	human umbilical vein endothelial cells
CAM	chorioallantoic membrane
IDP	ionic dissolution products
ATP	adenosine triphosphate

bFGF	basic fibroblast growth factor
VEGF	vascular endothelial growth factor
HIF-1α	hypoxia inducible factor-1α
PlGF	placental growth factor
EDN1	endothelin-1
NaBC1	Na^+-coupled borate transporter
BSP	bone sialoprotein
GPCRs	G protein coupled receptors
pH GPCRs	proton-sensitive GPCRs
OGR1/GPR68	ovarian cancer G protein-coupled receptor 1
TDAG8/GPR65	T cell death-related gene 8
EPCs	endothelial progenitor cells
cAMP	cyclic adenosine monophosphate
Rho	Ras homologous

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2025.03.006.

References

[1] M.N. Rahaman, D.E. Day, B.S. Bal, Q. Fu, S.B. Jung, L.F. Bonewald, A.P. Tomsia, Bioactive glass in tissue engineering, Acta Biomater. 7 (6) (2011) 2355–2373, https://doi.org/10.1016/j.actbio.2011.03.016.

[2] L.L. Hench, J.M. Polak, Third-generation biomedical materials, Science 295 (2002) 1014–1017, https://doi.org/10.1126/science.1067404.

[3] L.L. Hench, Biomaterials, Science 208 (4446) (1980) 826–831, https://doi.org/10.1126/science.6246576.

[4] L.L. Hench, Bioceramics: from concept to clinic, Am. Ceram. Soc. 74 (1991) 1487–1510, https://doi.org/10.1111/j.1151-2916.1991.tb07132.x.

[5] L.L. Hench, The story of Bioglass, J. Mater. Sci. Mater. Med. 17 (2006) 967–978, https://doi.org/10.1007/s10856-006-0432-z.

[6] W. Huang, D.E. Day, K. Kittiratanapiboon, M.N. Rahaman, Kinetics and mechanisms of the conversion of silicate (45S5), borate, and borosilicate glasses to hydroxyapatite in dilute phosphate solutions, J. Mater. Sci. Mater. Med. 17 (2006) 583–596, https://doi.org/10.1007/s10856-006-9220-z.

[7] A. Yao, D. Wang, W. Huang, Q. Fu, M.N. Rahaman, D.E. Day, In vitro bioactive characteristics of borate-based glasses with controllable degradation behavior, J. Am. Ceram. Soc. 90 (2007) 303–306, https://doi.org/10.1111/j.1551-2916.2006.01358.x.

[8] Q. Fu, M.N. Rahaman, H. Fu, X. Liu, Silicate, borosilicate, and borate bioactive glass scaffolds with controllable degradation rate for bone tissue engineering applications. I. Preparation and in vitro degradation, J, Biomed, Mater. Res. 95 (2010) 164–171, https://doi.org/10.1002/jbm.a.32824.

[9] Q. Fu, E. Saiz, M.N. Rahaman, A.P. Tomsia, Bioactive glass scaffolds for bone tissue engineering: state of the art and future perspectives, Mater. Sci. Eng. C 31 (7) (2011) 1245–1256, https://doi.org/10.1016/j.msec.2011.02.010.

[10] Y. Gu, W. Xiao, L. Lu, W. Huang, M.N. Rahaman, D. Wang, Kinetics and mechanisms of converting bioactive borate glasses to hydroxyapatite in aqueous phosphate solution, J. Mater. Sci. 46 (2011) 47–54, https://doi.org/10.1007/s10853-010-4792-x.

[11] X. Zhang, W. Jia, Y. Gu, W. Xiao, X. Liu, D. Wang, C. Zhang, W. Huang, M. N. Rahaman, D.E. Day, N. Zhou, Teicoplanin-loaded borate bioactive glass implants for treating chronic bone infection in a rabbit tibia osteomyelitis model, Biomaterials 31 (2010) 5865–5874, https://doi.org/10.1016/j.biomaterials.2010.04.005.

[12] D. Zhao, J. Yu, W. Huang, N. Zhou, D. Wang, W. Yin, Y. Chen, Dysprosium lithium borate glass mircrospheres for radiation synovectomy: the in vitro and in vivo performance evaluation, Mater. Sci. Eng. C. 30 (2010) 970–974, https://doi.org/10.1016/j.msec.2010.04.011.

[13] L.L. Hench, I.D. Xynos, J.M. Polak, Bioactive glasses for in situ tissue regeneration, J. Biomater. Sci. Polym. Ed. 15 (2004) 543–562, https://doi.org/10.1163/156856204323005352.

[14] X. Chen, Y. Meng, Y. Li, N. Zhao, Investigation on bio-mineralization of melt and sol–gel derived bioactive glasses, Appl. Surf. Sci. 255 (2008) 562–564, https://doi.org/10.1016/j.apsusc.2008.06.101.

[15] T. Chen, P. Du, X. Li, K. Su, S. Li, X. C, H. Pan, Osteogenic effect of borosilicate bioactive glass in vitro and in vivo, J. Chin. Ceram. Soc. 51 (10) (2023), https://doi.org/10.14062/j.issn.0454-5648.20230199.

[16] W. Zhang, F. Zhao, D. Huang, X. Fu, X. Li, X. Chen, Strontium-substituted submicrometer bioactive glasses modulate macrophage responses for improved bone regeneration, ACS Appl. Mater. Interfaces 8 (45) (2016) 30747–30758, https://doi.org/10.1021/acsami.6b10378.

[17] X. Li, X. Chen, G. Miao, H. Liu, C. Mao, G. Yuan, Q. Liang, X. Shen, C. Ning, X. Fu, Synthesis of radial mesoporous bioactive glass particles to deliver osteoactivin gene, J. Mater. Chem. B 2 (2014) 7045–7054, https://doi.org/10.1039/c4tb00883a.

[18] X. Li, L. Zhao, Q. Liang, J. Ye, N. Komatsu, Q. Zhang, W. Gao, M. Xu, X. Chen, Cationic polyarginine conjugated mesoporous bioactive glass nanoparticles with polyglycerol coating for efficient DNA delivery, J. Biomed. Nanotechnol. 13 (2017) 280–289, https://doi.org/10.1166/jbn.2017.2350.

[19] X. Li, Q. Liang, W. Zhang, Y. Li, J. Ye, F. Zhao, X. Chen, S. Wang, Bio-inspired bioactive glasses for efficient microRNA and drug delivery, J. Mater. Chem. B 5 (2017) 6376–6384, https://doi.org/10.1039/c7tb01021d.

[20] Q. Hu, Y. Li, N. Zhao, C. Ning, X. Chen, Facile synthesis of hollow mesoporous bioactive glass sub-micron spheres with a tunable cavity size, Mater. Lett. 134 (2014) 130–133, https://doi.org/10.1016/j.matlet.2014.07.041.

[21] N. Stone-Weiss, H. Bradtmüller, M. Fortino, M. Bertani, R.E. Youngman, A. Pedone, H. Eckert, A. Goel, Combined experimental and computational approach toward the structural design of borosilicate-based bioactive glasses, J. Phys. Chem. C 124 (2020) 17655–17674, https://doi.org/10.1021/acs.jpcc.0c04470.

[22] N. Stone-Weiss, H. Bradtmüller, H. Eckert, A. Goel, Composition-structure-solubility relationships in borosilicate glasses: toward a rational design of bioactive glasses with controlled dissolution behavior, ACS Appl. Mater. Interfaces 13 (2021) 31495–31513, https://doi.org/10.1021/acsami.1c07519.

[23] W. Jia, G.Y. Lau, W. Huang, C. Zhang, A.P. Tomsia, Q. Fu, Bioactive glass for large bone repair, Adv. Healthc. Mater. 4 (2015) 2842–2848, https://doi.org/10.1002/adhm.201500447.

[24] N. Stone-Weiss, E.M. Pierce, R.E. Youngman, O. Gulbiten, N.J. Smith, J. Du, A. Goel, Understanding the structural drivers governing glass-water interactions in borosilicate based model bioactive glasses, Acta, Biomater 65 (2018) 436–449, https://doi.org/10.1016/j.actbio.2017.11.006.

[25] J.L. George, R.K. Brow, In-situ characterization of borate glass dissolution kinetics by μ-Raman spectroscopy, J. Non-Cryst. Solids 426 (2015) 116–124, https://doi.org/10.1016/j.jnoncrysol.2015.07.003.col.

[26] K.A. Cole, G.A. Funk, M.N. Rahaman, T.E. McIff, Characterization of the conversion of bone cement and borate bioactive glass composites, J. Biomed. Mater. Res. B Appl. Biomater. 108 (2020) 1580–1591, https://doi.org/10.1002/jbm.b.34505.

[27] M. Sağlam, M. Hatipoğlu, S. Köseoğlu, H.H. Esen, S. Kelebek, Boric acid inhibits alveolar bone loss in rats by affecting RANKL and osteoprotegerin expression, J. Periodontal. Res. 49 (4) (2014) 472–479, https://doi.org/10.1111/jre.12127.

[28] S.S. Hakki, B.S. Bozkurt, E.E. Hakki, Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1), J. Trace Elem. Med. Biol. 24 (4) (2010) 243–250, https://doi.org/10.1016/j.jtemb.2010.04.001.

[29] U. Acaroz, S. Ince, D. Arslan-Acaroz, Z. Gurler, I. Kucukkurt, H.H. Demirel, H. O. Arslan, N. Varol, K. Zhu, The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats, Food Chem. Toxicol. 118 (2018) 745–752, https://doi.org/10.1016/j.fct.2018.06.031.

[30] A. Hoppe, N.S. Güldal, A.R. Boccaccini, A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics, Biomaterials 32 (2011) 2757–2774, https://doi.org/10.1016/j.biomaterials.2011.01.004.

[31] P. Valério, M. Pereira, A. Goes, BG60S dissolution interferes with osteoblast calcium signals, J. Mater. Sci. Mater. Med. 18 (1) (2007) 265–271, https://doi.org/10.1007/s10856-006-0697-3.

[32] C. Seaborn, F.H. Nielsen, Silicon deprivation decreases collagen formation in wounds and bone, and ornithine transaminase enzyme activity in liver, Biol. Trace Elem. Res. 89 (3) (2002) 251–261, https://doi.org/10.1385/BTER:89:3:251.

[33] O.D. Abodunrin, K. El Mabrouk, M. Bricha, A review on borate bioactive glasses (BBG): effect of doping elements, degradation, and applications, J. Mater. Chem. B 11 (2023) 955–973, https://doi.org/10.1039/d2tb02505a.

[34] O.D. Abodunrin, K. El Mabrouk, M. Bricha, Exploring the bioactivity and antibacterial properties of silver and cerium co-doped borosilicate bioactive glass, J. Mater. Chem. B (2025), https://doi.org/10.1039/D4TB02416H. Advance Article.

[35] L. Bai, P. Song, J. Su, Bioactive elements manipulate bone regeneration, Biomater. Transl. 4 (4) (2023) 248–269, https://doi.org/10.12336/biomatertransl.2023.04.005.

[36] W. Zhang, D. Huang, F. Zhao, W. Gao, L. Sun, X. Li, X. Chen, Synergistic effect of strontium and silicon in strontium-substituted sub-micron bioactive glass for enhanced osteogenesis, Mater. Sci. Eng C. Mater. Biol. Appl. 89 (2018) 245–255, https://doi.org/10.1016/j.msec.2018.04.012.

[37] H. Yuan, L. Niu, K. Jiao, D. Pei, C. Pramanik, J. Li, R. Messer, S. Kumar, D. H. Pashley, F.R. Tay, Revival of nitrogen-containing bisphosphonate-induced inhibition of osteoclastogenesis and osteoclast function by water-soluble microfibrous borate glass, Acta Biomater. 31 (2016) 312–325, https://doi.org/10.1016/j.actbio.2015.12.009.

[38] L. Chen, J. Song, S. Li, Y. Zhu, M. Liu, M. Wan, Z. Mu, F.R. Tay, L. Niu, Advances in materials-based therapeutic strategies against osteoporosis, Biomaterials 296 (2023) 122066, https://doi.org/10.1016/j.biomaterials.2023.122066.

[39] L. Bai, J. Su, Converging technologies in biomaterial translational research, Biomater. Transl. 4 (4) (2023) 197–198, https://doi.org/10.12336/biomatertransl.2023.04.001.

[40] M.T. Islam, L. Macri-Pellizzeri, V. Sottile, I. Ahmed, Rapid conversion of highly porous borate glass microspheres into hydroxyapatite, Biomater. Sci. 9 (2021) 1826–1844, https://doi.org/10.1039/d0bm01776k.

[41] H. Pan, X. Zhao, B.W. Darvell, W.W. Lu, Apatite-formation ability–predictor of "bioactivity"? Acta, Biomater 6 (2010) 4181–4188, https://doi.org/10.1016/j.actbio.2010.05.013.

[42] T.D. Pollard, W.C. Earnshaw, J. Lippincott-Schwartz, G.T. Johnson, Cell. Biology, Elsevier, 2017, pp. 525–541, https://doi.org/10.1016/C2014-0-00272-9.
[43] W.M. de Vos, F.A. Leermakers, A. de Keizer, M.A. Cohen Stuart, J.M. Kleijn, Field theoretical analysis of driving forces for the uptake of proteins by like-charged polyelectrolyte brushes: effects of charge regulation and patchiness, Langmuir 26 (2010) 249–259, https://doi.org/10.1021/la902079u.
[44] J. Will, L.C. Gerhardt, A.R. Boccaccini, Bioactive glass-based scaffolds for bone tissue engineering, Adv. Biochem. Eng. Biotechnol. 126 (2012) 195–226, https://doi.org/10.1007/10_2011_106.
[45] B. Lei, K.H. Shin, D.Y. Noh, I.H. Jo, Y.H. Koh, H.E. Kim, S.E. Kim, Sol-gel derived nanoscale bioactive glass (NBG) particles reinforced poly(ε-caprolactone) composites for bone tissue engineering, Mater. Sci. Eng. C. Mater. Biol. Appl. 33 (2013) 1102–1108, https://doi.org/10.1016/j.msec.2012.11.039.
[46] L.L. Hench, Bioceramics, J. Am. Ceram. Soc. 81 (2005) 1705–1728, https://doi.org/10.1111/j.1151-2916.1998.tb02540.x.
[47] G. Anand, S. Sharma, A.K. Dutta, S.K. Kumar, G. Belfort, Conformational transitions of adsorbed proteins on surfaces of varying polarity, Langmuir 26 (2010) 10803–10811, https://doi.org/10.1021/la1006132.
[48] P.E. Scopelliti, A. Borgonovo, M. Indrieri, L. Giorgetti, G. Bongiorno, R. Carbone, A. Podestà, P. Milani, The effect of surface nanometre-scale morphology on protein adsorption, PLoS One 5 (2010) e11862, https://doi.org/10.1371/journal.pone.0011862.
[49] K.C.R. Kolan, Y.W. Huang, J.A. Semon, M.C. Leu, 3D-printed biomimetic bioactive glass scaffolds for bone regeneration in rat calvarial defects, Int. J. Bioprint. 6 (2020) 274, https://doi.org/10.18063/ijb.v6i2.274.
[50] X. Cui, C. Huang, M. Zhang, C. Ruan, S. Peng, L. Li, W. Liu, T. Wang, B. Li, W. Huang, M.N. Rahaman, W.W. Lu, H. Pan, Enhanced osteointegration of poly (methylmethacrylate) bone cements by incorporating strontium-containing borate bioactive glass, J. R. Soc. Interface. 14 (2017) 20161057, https://doi.org/10.1098/rsif.2016.1057.
[51] H.T. Aiyelabegan, E. Sadroddiny, Fundamentals of protein and cell interactions in biomaterials, Biomed. Pharmacother. 88 (2017) 956–970, https://doi.org/10.1016/j.biopha.2017.01.136.
[52] P. Kanchanawong, D.A. Calderwood, Organization, dynamics and mechanoregulation of integrin-mediated cell-ECM adhesions, Nat. Rev. Mol. Cell Biol. 24 (2023) 142–161, https://doi.org/10.1038/s41580-022-00531-5.
[53] C. Gao, S. Peng, P. Feng, C. Shuai, Bone biomaterials and interactions with stem cells, Bone, Res. 5 (2017) 17059, https://doi.org/10.1038/boneres.2017.59.
[54] M.Y. Shie, S.J. Ding, Integrin binding and MAPK signal pathways in primary cell responses to surface chemistry of calcium silicate cements, Biomaterials 34 (2013) 6589–6606, https://doi.org/10.1016/j.biomaterials.2013.05.075.
[55] J. Li, J. Li, Y. Wei, N. Xu, J. Li, X. Pu, J. Wang, Z. Huang, X. Liao, G. Yin, Ion release behavior of vanadium-doped mesoporous bioactive glass particles and the effect of the released ions on osteogenic differentiation of BMSCs via the FAK/MAPK signaling pathway, J. Mater. Chem. B 9 (2021) 7848–7865, https://doi.org/10.1039/d1tb01479j.
[56] L.A. Haro Durand, G.E. Vargas, N.M. Romero, R. Vera-Mesones, J.M. Porto-López, A.R. Boccaccini, M.P. Zago, A. Baldi, A. Gorustovich, Angiogenic effects of ionic dissolution products released from a boron-doped 45S5 bioactive glass, J. Mater. Chem. B 3 (2015) 1142–1148, https://doi.org/10.1039/c4tb01840k.
[57] T.R. Arnett, Acidosis, hypoxia and bone, Arch. Biochem. Biophys. 503 (2010) 103–109, https://doi.org/10.1016/j.abb.2010.07.021.
[58] G. Walters, I. Pountos, P.V. Giannoudis, The cytokines and micro-environment of fracture haematoma: current evidence, J. Tissue. Eng. Regen. Med. 12 (2018) e1662–e1677, https://doi.org/10.1002/term.2593.
[59] K.K. Frick, D.A. Bushinsky, Chronic metabolic acidosis reversibly inhibits extracellular matrix gene expression in mouse osteoblasts, Am. J. Physiol. 275 (1998) F840–F847, https://doi.org/10.1152/ajprenal.1998.275.5.F840.
[60] K. Kato, I. Morita, Promotion of osteoclast differentiation and activation in spite of impeded osteoblast-lineage differentiation under acidosis: effects of acidosis on bone metabolism, Biosci. Trends. 7 (2013) 33–41, https://doi.org/10.5582/bst.2013.v7.1.33.
[61] D.K. Subrahmanyam, M. Vadivelan, S. Giridharan, N. Balamurugan, Wilson's disease - a rare cause of renal tubular acidosis with metabolic bone disease, Indian J. Nephrol. 24 (2014) 171–174, https://doi.org/10.4103/0971-4065.132017.
[62] G.H. Lee, J.D. Hwang, J.Y. Choi, H.J. Park, J.Y. Cho, K.W. Kim, H.J. Chae, H. R. Kim, An acidic pH environment increases cell death and pro-inflammatory cytokine release in osteoblasts: the involvement of BAX inhibitor-1, Int. J. Biochem. Cell Biol. 43 (2011) 1305–1317, https://doi.org/10.1016/j.biocel.2011.05.004.
[63] M. Harada, N. Udagawa, K. Fukasawa, B.Y. Hiraoka, M. Mogi, Inorganic pyrophosphatase activity of purified bovine pulp alkaline phosphatase at physiological pH, J. Dent. Res. 65 (1986) 125–127, https://doi.org/10.1177/00220345860650020601.
[64] Y.H. Shen, W.C. Liu, C.Y. Wen, H.B. Pan, T. Wang, B.W. Darvell, W.W. Lu, W. H. Huang, Bone regeneration: importance of local pH—strontium-doped borosilicate scaffold, J. Mater. Chem 22 (2012) 8662–8670, https://doi.org/10.1039/c2jm16141a.
[65] M. Kimura, T. Sase, A. Higashikawa, M. Sato, T. Sato, M. Tazaki, Y. Shibukawa, High pH-sensitive TRPA1 activation in odontoblasts regulates mineralization, J. Dent. Res. 95 (2016) 1057–1064, https://doi.org/10.1177/0022034516644702.
[66] W. Liu, X. Dan, W.W. Lu, X. Zhao, C. Ruan, T. Wang, X. Cui, X. Zhai, Y. Ma, D. Wang, W. Huang, H. Pan, Spatial distribution of biomaterial microenvironment pH and its modulatory effect on osteoclasts at the early stage of bone defect regeneration, ACS Appl. Mater. Interfaces 11 (2019) 9557–9572, https://doi.org/10.1021/acsami.8b20580.
[67] X. Cui, W. Huang, Y. Zhang, C. Huang, Z. Yu, L. Wang, W. Liu, T. Wang, J. Zhou, H. Wang, N. Zhou, D. Wang, H. Pan, M.N. Rahaman, Evaluation of an injectable bioactive borate glass cement to heal bone defects in a rabbit femoral condyle model, Mater. Sci. Eng C. Mater. Biol. Appl. 73 (2017) 585–595, https://doi.org/10.1016/j.msec.2016.12.101.
[68] Y. Zhu, Y. Ouyang, Y. Chang, C. Luo, J. Xu, C. Zhang, W. Huang, Evaluation of the proliferation and differentiation behaviors of mesenchymal stem cells with partially converted borate glass containing different amounts of strontium in vitro, Mol. Med. Rep. 7 (2013) 1129–1136, https://doi.org/10.3892/mmr.2013.1341.
[69] J. Zhang, J. Guan, C. Zhang, H. Wang, W. Huang, S. Guo, X. Niu, Z. Xie, Y. Wang, Bioactive borate glass promotes the repair of radius segmental bone defects by enhancing the osteogenic differentiation of BMSCs, Biomed. Mater. 10 (2015) 065011, https://doi.org/10.1088/1748-6041/10/6/065011.
[70] S. Verrier, J.J. Blaker, V. Maquet, L.L. Hench, A.R. Boccaccini, PDLLA/Bioglass composites for soft-tissue and hard-tissue engineering: an in vitro cell biology assessment, Biomaterials 25 (2004) 3013–3021, https://doi.org/10.1016/j.biomaterials.2003.09.081.
[71] A. Oliva, A. Salerno, B. Locardi, V. Riccio, F. Della Ragione, P. Iardino, V. Zappia, Behaviour of human osteoblasts cultured on bioactive glass coatings, Biomaterials 19 (1998) 1019–1025, https://doi.org/10.1016/s0142-9612(97)00249-4.
[72] K.F. Bombonato-Prado, L.S. Bellesini, C.M. Junta, M.M. Marques, G.A. Passos, A. L. Rosa, Microarray-based gene expression analysis of human osteoblasts in response to different biomaterials, J. Biomed. Mater. Res. A. 88 (2009) 401–408, https://doi.org/10.1002/jbm.a.31701.
[73] I.D. Xynos, A.J. Edgar, L.D. Buttery, L.L. Hench, J.M. Polak, Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution, J. Biomed. Mater. Res. 55 (2001) 151–157, https://doi.org/10.1002/1097-4636(200105)55:2<151::aid-jbm1001>3.0.co;2-d.
[74] I.D. Xynos, M.V. Hukkanen, J.J. Batten, L.D. Buttery, L.L. Hench, J.M. Polak, Bioglass 45S5 stimulates osteoblast turnover and enhances bone formation in vitro: implications and applications for bone tissue engineering, Calcif. Tissue, Int 67 (2000) 321–329, https://doi.org/10.1007/s002230001134.
[75] P. Valerio, M.M. Pereira, A.M. Goes, M.F. Leite, The effect of ionic products from bioactive glass dissolution on osteoblast proliferation and collagen production, Biomaterials 25 (2004) 2941–2948, https://doi.org/10.1016/j.biomaterials.2003.09.086.
[76] C. Wu, R. Miron, A. Sculean, S. Kaskel, T. Doert, R. Schulze, Y. Zhang, Proliferation, differentiation and gene expression of osteoblasts in boron-containing associated with dexamethasone deliver from mesoporous bioactive glass scaffolds, Biomaterials 32 (2011) 7068–7078, https://doi.org/10.1016/j.biomaterials.2011.06.009.
[77] L. Xia, W. Ma, Y. Zhou, Z. Gui, A. Yao, D. Wang, A. Takemura, M. Uemura, K. Lin, Y. Xu, Stimulatory effects of boron containing bioactive glass on osteogenesis and angiogenesis of polycaprolactone: in vitro study, BioMed Res. Int. (2019) 8961409, https://doi.org/10.1155/2019/8961409.
[78] A. Szczodra, A. Houaoui, R. Agniel, L. Sicard, S. Miettinen, J. Massera, C. Gorin, Boron substitution in silicate bioactive glass scaffolds to enhance bone differentiation and regeneration, Acta, Biomater 186 (2024) 489–506, https://doi.org/10.1016/j.actbio.2024.07.053.
[79] L.A. Fiehn, E. Kunisch, M. Saur, M. Arango-Ospina, C. Merle, S. Hagmann, A. Stiller, L. Hupa, H. Kaňková, D. Galusková, T. Renkawitz, A.R. Boccaccini, F. Westhauser, A comparative in vitro and in vivo analysis of the impact of copper substitution on the cytocompatibility, osteogenic, and angiogenic properties of a borosilicate bioactive glass, J. Biomed. Mater. Res. A. 112 (2024) 1740–1759, https://doi.org/10.1002/jbm.a.37721.
[80] Y. Li, J. Yue, Y. Liu, J. Wu, M. Guan, D. Chen, H. Pan, X. Zhao, W.W. Lu, Strontium regulates stem cell fate during osteogenic differentiation through asymmetric cell division, Acta, Biomater 119 (2021) 432–443, https://doi.org/10.1016/j.actbio.2020.10.030.
[81] R.F. Brown, M.N. Rahaman, A.B. Dwilewicz, W. Huang, D.E. Day, Y. Li, B.S. Bal, Effect of borate glass composition on its conversion to hydroxyapatite and on the proliferation of MC3T3-E1 cells, J. Biomed. Mater. Res. A. 88 (2009) 392–400, https://doi.org/10.1002/jbm.a.31679.
[82] M. Ojansivu, A. Mishra, S. Vanhatupa, M. Juntunen, A. Larionova, J. Massera, S. Miettinen, The effect of S53P4-based borosilicate glasses and glass dissolution products on the osteogenic commitment of human adipose stem cells, PLoS One 13 (2018) e0202740, https://doi.org/10.1371/journal.pone.0202740.
[83] R. Baron, M. Kneissel, WNT signaling in bone homeostasis and disease: from human mutations to treatments, Nat. Med. 19 (2013) 179–192, https://doi.org/10.1038/nm.3074.
[84] X. Cui, Y. Zhang, J. Wang, C. Huang, Y. Wang, H. Yang, W. Liu, T. Wang, D. Wang, G. Wang, C. Ruan, D. Chen, W.W. Lu, W. Huang, M.N. Rahaman, H. Pan, Strontium modulates osteogenic activity of bone cement composed of bioactive borosilicate glass particles by activating Wnt/β-catenin signaling pathway, Bioact. Mater. 5 (2020) 334–347, https://doi.org/10.1016/j.bioactmat.2020.02.016.
[85] C. Yin, X. Jia, R.J. Miron, Q. Long, H. Xu, Y. Wei, M. Wu, Y. Zhang, Z. Li, Setd7 and its contribution to Boron-induced bone regeneration in Boron-mesoporous bioactive glass scaffolds, Acta, Biomater 73 (2018) 522–530, https://doi.org/10.1016/j.actbio.2018.04.033.
[86] C. Yin, X. Jia, Q. Zhao, Z. Zhao, J. Wang, Y. Zhang, Z. Li, H. Sun, Z. Li, Transcription factor 7-like 2 promotes osteogenic differentiation and boron-

induced bone repair via lipocalin 2, Mater. Sci. Eng C. Mater. Biol. Appl. 110 (2020) 110671, https://doi.org/10.1016/j.msec.2020.110671.
[87] M.B. Greenblatt, J.H. Shim, L.H. Glimcher, Mitogen-activated protein kinase pathways in osteoblasts, Annu. Rev. Cell Dev. Biol. 29 (2013) 63–79, https://doi.org/10.1146/annurev-cellbio-101512-122347.
[88] I. Christodoulou, L.D. Buttery, G. Tai, L.L. Hench, J.M. Polak, Characterization of human fetal osteoblasts by microarray analysis following stimulation with 58S bioactive gel-glass ionic dissolution products, J. Biomed. Mater. Res. B Appl. Biomater. 77 (2006) 431–446, https://doi.org/10.1002/jbm.b.30455.
[89] K. Gao, X. Wang, Z. Wang, L. He, J. Lin, Z. Bai, K. Jiang, S. Huang, W. Zheng, L. Liu, Design of novel functionalized collagen-chitosan-MBG scaffolds for enhancing osteoblast differentiation in BMSCs, Biomed. Mater. 16 (2021) 065028, https://doi.org/10.1088/1748-605X/ac3146.
[90] A.Y. Au, R.Y. Au, J.L. Demko, R.M. McLaughlin, B.E. Eves, C.G. Frondoza, Consil bioactive glass particles enhance osteoblast proliferation and selectively modulate cell signaling pathways in vitro, J. Biomed. Mater. Res. A. 94 (2010) 380–388, https://doi.org/10.1002/jbm.a.32668.
[91] D.Y. Shu, F.J. Lovicu, Insights into bone morphogenetic protein-(BMP-) signaling in ocular lens biology and pathology, Cells 10 (2021) 2604, https://doi.org/10.3390/cells10102604.
[92] Y. Zhang, P.A. Beachy, Cellular and molecular mechanisms of Hedgehog signaling, Nat. Rev. Mol. Cell Biol. 24 (2023) 668–687, https://doi.org/10.1038/s41580-023-00591-1.
[93] D.F. Williams, Biocompatibility pathways and mechanisms for bioactive materials: the bioactivity zone, Bioact. Mater. 10 (2021) 306–322, https://doi.org/10.1016/j.bioactmat.2021.08.014.
[94] A. Asselin, S. Hattar, M. Oboeuf, D. Greenspan, A. Berdal, J.M. Sautier, The modulation of tissue-specific gene expression in rat nasal chondrocyte cultures by bioactive glasses, Biomaterials 25 (2004), https://doi.org/10.1016/j.biomaterials.2004.01.016, 5621-5430.
[95] C. Zhang, Y. Yuan, L. Fang, Y. Xuan, Promotion of osteogenesis by bioactive glass-ceramic coating: possible involvement of the Hedgehog signaling pathway, J. Orthop. Sci. 24 (2019) 731–736, https://doi.org/10.1016/j.jos.2018.12.006.
[96] D. Huang, F. Zhao, W. Gao, X. Chen, Z. Guo, W. Zhang, Strontium-substituted sub-micron bioactive glasses inhibit ostoclastogenesis through suppression of RANKL-induced signaling pathway, Regen. Biomater. 7 (2020) 303–311, https://doi.org/10.1093/rb/rbaa004.
[97] C. Pan, L. Chen, R. Wu, H. Shan, Z. Zhou, Y. Lin, X. Yu, L. Yan, C. Wu, Lithium-containing biomaterials inhibit osteoclastogenesis of macrophages in vitro and osteolysis in vivo, J. Mater. Chem. B 6 (2018) 8115–8126, https://doi.org/10.1039/c8tb02678e.
[98] H. Zhang, Y. Cui, X. Zhuo, J. Kim, H. Li, S. Li, H. Yang, K. Su, C. Liu, P. Tian, X. Li, L. Li, D. Wang, L. Zhao, J. Wang, X. Cui, B. Li, H. Pan, Biological fixation of bioactive bone cement in vertebroplasty: the first clinical investigation of borosilicate glass (BSG) reinforced PMMA bone cement, ACS Appl. Mater. Interfaces 14 (2022) 51711–51727, https://doi.org/10.1021/acsami.2c15250.
[99] R. Vlashi, X. Zhang, M. Wu, G. Chen, Wnt signaling: essential roles in osteoblast differentiation, bone metabolism and therapeutic implications for bone and skeletal disorders, Genes, Dis 10 (2022) 1291–1317, https://doi.org/10.1016/j.gendis.2022.07.011.
[100] M. Ojansivu, L. Hyväri, M. Kellomäki, L. Hupa, S. Vanhatupa, S. Miettinen, Bioactive glass induced osteogenic differentiation of human adipose stem cells is dependent on cell attachment mechanism and mitogen-activated protein kinases, Eur. Cell. Mater. 35 (2018) 54–72, https://doi.org/10.22203/eCM.v035a05.
[101] Y. Zhang, T. Pizzute, M. Pei, A review of crosstalk between MAPK and Wnt signals and its impact on cartilage regeneration, Cell Tissue Res. 358 (2014) 633–649, https://doi.org/10.1007/s00441-014-2010-x.
[102] Y. Chen, G. Whetstone, E.E. Lin, J.E. Nadesan, J. Wei, J. Poon, B.A. Alman, β-Catenin signaling pathway is crucial for bone morphogenetic protein 2 to induce new bone formation, J. Biol. Chem. 282 (1) (2007) 526–533, https://doi.org/10.1074/jbc.M602700200.
[103] J. Chang, F. Liu, M. Lee, B. Wu, K. Ting, J.N. Zara, C. Soo, K. Al Hezaimi, W. Zou, X. Chen, D.J. Mooney, C.-Y. Wang, NF-κB inhibits osteogenic differentiation of mesenchymal stem cells by promoting β-catenin degradation, Proc. Natl. Acad. Sci. USA. 110 (23) (2013) 9469–9474, https://doi.org/10.1073/pnas.1300532110.
[104] J. Guo, F. Wang, Y. Hu, Y. Luo, Y. Wei, K. Xu, H. Zhang, H. Liu, L. Bo, S. Lv, S. Sheng, X. Zhuang, T. Zhang, C. Xu, X. Chen, J. Su, Exosome-based bone-targeting drug delivery alleviates impaired osteoblastic bone formation and bone loss in inflammatory bowel diseases, Cell Rep. Med. 4 (1) (2023) 100881, https://doi.org/10.1016/j.xcrm.2022.100881.
[105] H. Qiu, H. Xiong, J. Zheng, Y. Peng, C. Wang, Q. Hu, F. Zhao, K. Chen, Sr-incorporated bioactive glass remodels the immunological microenvironment by enhancing the mitochondrial function of macrophage via the PI3K/AKT/mTOR signaling pathway, ACS Biomater. Sci. Eng. 10 (2024) 3923–3934, https://doi.org/10.1021/acsbiomaterials.4c00228.
[106] R. Lin, C. Deng, X. Li, Y. Liu, M. Zhang, C. Qin, Q. Yao, L. Wang, C. Wu, Copper-incorporated bioactive glass-ceramics inducing anti-inflammatory phenotype and regeneration of cartilage/bone interface, Theranostics 9 (2019) 6300–6313, https://doi.org/10.7150/thno.36120.
[107] F. Martin, T. Linden, D.M. Katschinski, F. Oehme, I. Flamme, C. K. Mukhopadhyay, K. Eckhardt, J. Tröger, S. Barth, G. Camenisch, R.H. Wenger, Copper-dependent activation of hypoxia-inducible factor (HIF)-1: implications for ceruloplasmin regulation, Blood 105 (12) (2005) 4613–4619, https://doi.org/10.1182/blood-2004-10-3980.
[108] E.M. O'Brien, G.E. Risser, K.L. Spiller, Sequential drug delivery to modulate macrophage behavior and enhance implant integration, Adv. Drug Deliv. Rev. 149–150 (2019) 85–94, https://doi.org/10.1016/j.addr.2019.05.005.
[109] C.Z.W. Lee, T. Kozaki, F. Ginhoux, Studying tissue macrophages in vitro: are iPSC-derived cells the answer? Nat. Rev. Immunol. 18 (2018) 716–725, https://doi.org/10.1038/s41577-018-0054-y.
[110] B.N. Brown, B.D. Ratner, S.B. Goodman, S. Amar, S.F. Badylak, Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine, Biomaterials 33 (2012) 3792–3802, https://doi.org/10.1016/j.biomaterials.2012.02.034.
[111] P.J. Murray, Macrophage polarization, Annu. Rev. Physiol. 79 (2017) 541–566, https://doi.org/10.1146/annurev-physiol-022516-034339.
[112] J.R. Alhamdi, T. Peng, I.M. Al-Naggar, K.L. Hawley, K.L. Spiller, L.T. Kuhn, Controlled M1-to-M2 transition of aged macrophages by calcium phosphate coatings, Biomaterials 196 (2019) 90–99, https://doi.org/10.1016/j.biomaterials.2018.07.012.
[113] Z. Chen, C. Wu, W. Gu, T. Klein, R. Crawford, Y. Xiao, Osteogenic differentiation of bone marrow MSCs by β-tricalcium phosphate stimulating macrophages via BMP2 signalling pathway, Biomaterials 35 (2014) 1507–1518, https://doi.org/10.1016/j.biomaterials.2013.11.014.
[114] R.M. Day, A.R. Boccaccini, Effect of particulate bioactive glasses on human macrophages and monocytes in vitro, J. Biomed. Mater. Res. A. 73 (2005) 73–79, https://doi.org/10.1002/jbm.a.30262.
[115] M.J. Feito, L. Casarrubios, M. Oñaderra, M. Gómez-Duro, P. Arribas, A. Polo-Montalvo, M. Vallet-Regí, D. Arcos, M.T. Portolés, Response of RAW 264.7 and J774A.1 macrophages to particles and nanoparticles of a mesoporous bioactive glass: a comparative study, Colloids Surf. B Biointerfaces 208 (2021) 112110, https://doi.org/10.1016/j.colsurfb.2021.112110.
[116] M. Barbeck, S. Alkildani, A. Mandlule, M. Radenković, S. Najm, In vivo analysis of the immune response to strontium- and copper-doped bioglass, In Vivo (Athens) 36 (5) (2022) 2149–2165, https://doi.org/10.21873/invivo.12941.
[117] B.D. Manning, A. Toker, AKT/PKB signaling: navigating the network, Cell 169 (3) (2017) 381–405, https://doi.org/10.1016/j.cell.2017.04.001.
[118] R.A. Saxton, D.M. Sabatini, mTOR signaling in growth, metabolism, and disease, Cell 168 (6) (2017) 960–976, https://doi.org/10.1016/j.cell.2017.02.004.
[119] S. Li, L. Zhang, C. Liu, J. Kim, K. Su, T. Chen, L. Zhao, X. Lu, H. Zhang, Y. Cui, X. Cui, F. Yuan, H. Pan, Spontaneous immunomodulation and regulation of angiogenesis and osteogenesis by Sr/Cu-borosilicate glass (BSG) bone cement to repair critical bone defects, Bioact. Mater. 23 (2022) 101–117, https://doi.org/10.1016/j.bioactmat.2022.10.021.
[120] P. Tian, L. Zhao, J. Kim, X. Li, C. Liu, X. Cui, T. Liang, Y. Du, X. Chen, H. Pan, Dual stimulus responsive borosilicate glass (BSG) scaffolds promote diabetic alveolar bone defectsrepair by modulating macrophage phenotype, Bioact. Mater. 26 (2023) 231–248, https://doi.org/10.1016/j.bioactmat.2023.02.023.
[121] J. Ding, J. Zhao, L. Wang, X. Chen, D. Jiang, M. Qin, Z. Zhu, D. Wang, W. Jia, Regulated contribution of local and systemic immunity to new bone regeneration by modulating B/Sr concentration of bioactive borosilicate glass, Mater. Today. Bio. 19 (2023) 100585, https://doi.org/10.1016/j.mtbio.2023.100585.
[122] S. Decker, M. Arango-Ospina, F. Rehder, A. Moghaddam, R. Simon, C. Merle, T. Renkawitz, A.R. Boccaccini, F. Westhauser, In vitro and in ovo impact of the ionic dissolution products of boron-doped bioactive silicate glasses on cell viability, osteogenesis and angiogenesis, Sci. Rep. 12 (2022) 8510, https://doi.org/10.1038/s41598-022-12430-y.
[123] L.A. Haro Durand, A. Góngora, J.M. Porto López, A.R. Boccaccini, M.P. Zago, A. Baldi, A. Gorustovich, In vitro endothelial cell response to ionic dissolution products from boron-doped bioactive glass in the SiO2-CaO-P2O5-Na2O system, J. Mater. Chem. B 2 (2014) 7620–7630, https://doi.org/10.1039/c4tb01043d.
[124] E. Kunisch, L.A. Fiehn, M. Saur, M. Arango-Ospina, C. Merle, S. Hagmann, A. Stiller, L. Hupa, T. Renkawitz, A.R. Boccaccini, F. Westhauser, A comparative in vitro and in vivo analysis of the biological properties of the 45S5-, 1393-, and 0106-B1-bioactive glass compositions using human bone marrow-derived stromal cells and a rodent critical size femoral defect model, Biomater. Adv. 153 (2023) 213521, https://doi.org/10.1016/j.bioadv.2023.213521.
[125] F. Westhauser, B. Widholz, Q. Nawaz, S. Tsitlakidis, S. Hagmann, A. Moghaddam, A.R. Boccaccini, Favorable angiogenic properties of the borosilicate bioactive glass 0106-B1 result in enhanced in vivo osteoid formation compared to 45S5 Bioglass, Biomater. Sci. 7 (2019) 5161–5176, https://doi.org/10.1039/c9bm01220f.
[126] L.L. Hench, J.R. Jones, Bioactive glasses: frontiers and challenges, Front. Bioeng. Biotechnol. 3 (2015) 194, https://doi.org/10.3389/fbioe.2015.00194.
[127] F. Fayyazbakhsh, M.H. Tusar, Y.-W. Huang, M.C. Leu, Effect of bioactive borate glass on printability and physical properties of hydrogels, Mater. Sci. Addit. Manuf. 3 (1) (2024), https://doi.org/10.36922/msam.2845. Article No. 2845.
[128] S. Bose, S. Vahabzadeh, A. Bandyopadhyay, Bone tissue engineering using 3D printing, Mater. Today 16 (12) (2013) 496–504, https://doi.org/10.1016/j.mattod.2013.11.017.
[129] I. Uluisik, H.C. Karakaya, A. Koc, The importance of boron in biological systems, J. Trace, Elem. Med. Biol. 45 (2018) 156–162, https://doi.org/10.1016/j.jtemb.2017.10.008.
[130] M. Park, Q. Li, N. Shcheynikov, W. Zeng, S. Muallem, NaBC1 is a ubiquitous electrogenic Na+ -coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation, Mol. Cell. 16 (2004) 331–341, https://doi.org/10.1016/j.molcel.2004.09.030.
[131] K. Zhang, A. Alaohali, N. Sawangboon, P.T. Sharpe, D.S. Brauer, E. Gentleman, A comparison of lithium-substituted phosphate and borate bioactive glasses for

mineralised tissue repair, Dent. Mater. 35 (2019) 919–927, https://doi.org/10.1016/j.dental.2019.03.008.

[132] L. Deilmann, O. Winter, B. Cerrutti, H. Bradtmüller, C. Herzig, A. Limbeck, O. Lahayne, C. Hellmich, H. Eckert, D. Eder, Effect of boron incorporation on the bioactivity, structure, and mechanical properties of ordered mesoporous bioactive glasses, J. Mater. Chem. B 8 (2020) 1456–1465, https://doi.org/10.1039/c9tb01805k.

[133] K.T. Weiß, M. Fante, G. Köhl, J. Schreml, F. Haubner, M. Kreutz, S. Haverkampf, M. Berneburg, S. Schreml, Proton-sensing G protein-coupled receptors as regulators of cell proliferation and migration during tumor growth and wound healing, Exp. Dermatol. 26 (2017) 127–132, https://doi.org/10.1111/exd.13209.

[134] K.K. Frick, N.S. Krieger, K. Nehrke, D.A. Bushinsky, Metabolic acidosis increases intracellular calcium in bone cells through activation of the proton receptor OGR1, J. Bone Miner. Res. 24 (2009) 305–313, https://doi.org/10.1359/jbmr.081015.

[135] H. Tomura, J.Q. Wang, J.P. Liu, M. Komachi, A. Damirin, C. Mogi, M. Tobo, H. Nochi, K. Tamoto, D.S. Im, K. Sato, F. Okajima, Cyclooxygenase-2 expression and prostaglandin E2 production in response to acidic pH through OGR1 in a human osteoblastic cell line, J. Bone Miner. Res. 23 (2008) 1129–1139, https://doi.org/10.1359/jbmr.080236.