

REVIEW

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Tailoring of bioactive glass and glass-ceramics properties for *in vitro* and *in vivo* response optimization: a review

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Bioactive glasses are inorganic biocompatible materials that can find applications in many biomedical fields. The main application is bone and dental tissue engineering. However, some applications in contact with soft tissues are emerging. It is well known that both bulk (such as composition) and surface properties (such as morphology and wettability) of an implanted material influence the response of cells in contact with the implant. This review aims to elucidate and compare the main strategies that are employed to modulate cell behavior in contact with bioactive glasses. The first part of this review is focused on the doping of bioactive glasses with ions and drugs, which can be incorporated into the bio-ceramic to impart several therapeutic properties, such as osteogenic, proangiogenic, or/and antibacterial ones. The second part of this review is devoted to the chemical functionalization of bioactive glasses using drugs, extra-cellular matrix proteins, vitamins, and polyphenols. In the third and final part, the physical modifications of the surfaces of bioactive glasses are reviewed. Both top-down (removing materials from the surface, for example using laser treatment and etching strategies) and bottom-up (depositing materials on the surface, for example through the deposition of coatings) strategies are discussed.

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Introduction

Bioactive glasses (BGs) are a particular class of ceramics characterized by the well-known ability to interact and bond with hard tissues and, in some cases, also with soft tissues.^{1,2} The term bioactivity means the ability of a material to be active biologically, evoking a specific biological response that results in a bond between the material itself and a tissue.³ In particular, bioactive glasses fulfill their bioactivity through specific surface reactions that lead to the development of a silica gel layer that gradually promotes the formation of a layer of a biologically active mineral similar to hydroxyapatite (HA), generally called the HA-like layer, in the presence of body fluids *in vitro* or *in vivo*.³ Thanks to its chemical and structural similarity to the mineral constituent of bone, the newly formed HA can bond firmly with living bone and surrounding tissues, providing an interfacial bond between the implant and the body tissue.¹ Moreover, while degrading at a controllable rate and converting to HA-like material, BGs release ions in a controlled manner.⁴

In recent years, regenerative medicine and tissue engineering (TE) have emerged as innovative promising approaches for the repair and regeneration of lost or damaged tissues and organs.⁵ They have the potential to overcome the problems of

the shortage of donor tissues and organs available for transplantation and possible donor site morbidity,^{2,6,7} thanks to the development of new artificial materials and scaffolds, that are able to promote cell proliferation and tissue growth *in vitro* and *in vivo*.⁶

The clinical need for engineered bone tissue is still challenging in the field of orthopedic and craniofacial surgery, in direct relationship to the increasing human population and numerousness of traumatic injuries and pathological diseases, that can impair normal bone functions and lead to bone fractures non-unions, immobility, severe pain and deformity.⁷ Nowadays, the demand for bone grafts is very high and represents the second most common tissue transplantation procedure after blood. Each year there are approximately 1 million cases of skeletal defects requiring bone graft procedures to achieve union, with significant socioeconomic consequences, and over 2.2 million bone graft procedures are conducted worldwide annually in orthopedics and dentistry.⁷

The human skeleton has exceptional healing ability, often with absolute functional and structural recovery, but in some cases, both external and biological factors can destroy the natural regenerative processes of bone.⁷ Moreover, there is a critical size defect, beyond which a bone injury cannot be healed only by the natural bone healing capacity.⁷ For example, in the case of extensive bone defects (such as bone fracture, that require the reconstruction of large bone segments), the bone self-healing mechanism is not feasible.⁸

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Regeneration of large-size bone defects is clinically challenging and requires the use of multifunctional biomaterial, able to stimulate osteogenesis and angiogenesis and to obstacle bacterial infections.⁹

For a successful long-term implant, a good osseointegration is fundamental. This concept was first forwarded by Brånemark to describe a “direct structural and functional connection between ordered, living bone, and the surface of a load-carrying implant”.¹⁰ Osseointegration of implants is dependent on the attachment and proliferation of osteogenic cells on the implant surface, associated with the maturation and mineralization of their extracellular matrix (ECM).¹¹

When an implant is inserted into the body, a complex series of events occur at its surface, leading to the adsorption of water molecules and proteins, that mediate the subsequent cellular adhesion and the activation and release of cytokines and other soluble growth/differentiation factors.¹⁰ In the case of a bone implant, the activated osteogenic processes are very similar to those of bone healing,¹⁰ involving multiple stimuli, both physical (such as substrate topography, stiffness, shear stress, and electrical forces) and biochemical (such as growth factors, cytokines, genes or proteins) factors.⁷ Although the precise molecular mechanisms of osseointegration are still not totally understood, it is clear that the chemical and physical properties of the implant surface play a key role in the interactions between the implant and the host tissue, through the modulation of cell behavior, growth factor (GF) production and osteogenic gene expression.^{10,12} Hence, to stimulate bone regeneration and osseointegration, BGs are designed to determine cell gene expression by four main mechanisms: (1) surface chemistry, (2) topography, (3) rate and type of dissolution of the released ions, and (4) shear stress at scaffold/bone interfaces (by *ad hoc* tailoring of their mechanical properties).⁶

Furthermore, the host immune system strongly interacts with the implanted biomaterial, producing various inflammatory and anti-inflammatory cytokines and exploiting strong paracrine effects that influence cellular activities, in a cross-talk between immune cells and all other cells involved in the bone regeneration process, although during bone tissue healing individual cell types play important roles even independently.¹³ Therefore, the synthesis of BGs with anti-inflammatory properties could be useful to control post-implant inflammation and stimulate bone regeneration.

Recently, bioactive ceramics have been also applied in soft TE, in particular when regeneration of tissues at the interface with bone is required. Among these tissues, cartilage regeneration is widely studied^{14,15} because the damage of the joints' articular cartilage such as the hip and knee is very common and can occur as a consequence of both degenerative diseases, such as osteoarthritis, and trauma, for example in sports injuries.¹⁶ In these conditions, cartilage-engineered regeneration is of enormous benefit clinically.¹⁶ Although, nowadays, there is no evidence of cartilage growth on a BG surface, the formation of a new subchondral bone layer seems to support cartilage-like tissue, at least at the extremities of osteochondral defects.¹⁷ Therefore, for traumatic cartilage lesions and *osteo-*

chondritis dissecans, the initial repair of subchondral bone could be a potentially advantageous approach for cartilage healing.¹⁷ A solid surface or a support on which cartilage cells can spread is essential for the repair of cartilage in the case of large osteochondral defects.¹⁷

Pro-angiogenic properties are important not only in the case of soft TE and the regeneration of interfacial tissues but in tissue engineering in general, because a capillary/blood vessel network is a key requirement for blood supply and consequently oxygen and nutrient supply for the growing healing tissues, including bone tissue. Nowadays, the amount of oxygen required for cell survival in a tridimensional scaffold is limited to a diffusion distance between 150 and 200 μm from the supplying blood vessels.¹⁸ For the *in vivo* survival of a tissue-engineered construct with a size larger than this oxygen diffusion limit, the tissue has to be vascularized and possess a capillary network for the delivery of oxygen and nutrients to the cells within the construct.¹⁹ Thus, for a better regeneration of large bone defects, bioactive scaffolds must possess not only osteoconductivity (for the guidance of new bone growth), but also the ability to stimulate both osteogenesis (for promoting new bone formation) and angiogenesis (for inducing vascularization).²⁰ For example, in the case of highly vascularized bone, the lack of a functional microvasculature connected to the host blood supply has been identified as the culprit for implant failure.⁶ In the case of large osseous defects, if nourishment and oxygen transport are insufficient, due to lacking angiogenesis, bone tissue can generally grow only up to a thickness of just 150–200 μm (corresponding to the diffusion limit of solutes and gases inside the tissue from the surface) before facing necrosis.^{21,22}

Another critical issue related to bone implants is the risk of septic loosening. Unfortunately, despite the benefits of these artificial tissue substitutes and the strict antiseptic operative procedures, the problem of infections is still not solved²³ and the cells have to compete with bacteria to colonize the implant surface, in the so-called “race for the surface”.¹⁰ During the insertion of an implant, bacteria from the patient's own skin and/or mucosa enter the wound site, leading to implant-related infections, which are one of the most common reasons for surgical failure (14–29% of total failures), leading to implant removal.²³ This problem becomes even more critical when infections are caused by multidrug-resistant (MDR) pathogens.^{10,24} Most infections occur in the form of biofilms, and hence they are extremely resistant to host defenses and therapy.²⁵ Hospital-acquired infections are, generally, considered to be the third-largest cause affecting public health.¹⁰ Just a few data to highlight the gravity of this problem: in 2011, about 722 000 people in the United States (U.S.) were estimated to be involved in medical-device-related infections each year while residing in a U.S. hospital, and among them, about 75 000 patients died due to these infections;²⁶ whereas in Europe, each year more than 4.2 million patients are affected by bacterial infections acquired in hospitals.²⁶ Nowadays, the mortality rate of patients undergoing primary implant infections ranges from 10 to 18%. In the case of revised implants, this mortality rate can double or even

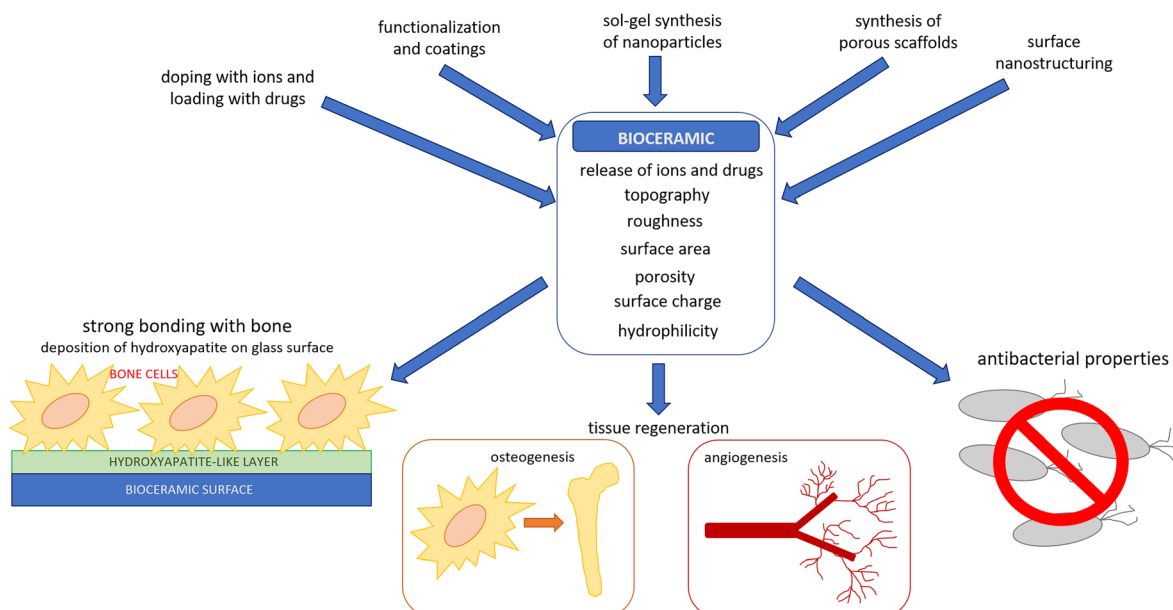


Therefore, artificial implants have to be designed *ad hoc*, so that they can stimulate tissue regeneration and hinder bacterial colonization. In this context, for the fabrication of engineered biomaterials, it is important to point out that all types of cell (both mammalian and bacterial cells) are inherently sensitive to local chemical and topographical mesoscale, microscale, and nanoscale patterns, so that the topography, mor-

To write the actual review, a literature search was performed using PICO (the discovery tool of Politecnico di Torino), Google Scholar, and PubMed. These electronic databases were examined using different combinations of the following keywords: “glass”, “ceramic”, “bioactive”, “bioceramic”, “doping”, “doped”, “ion effect”, “functionalization”, “coating”, “scaffold”, “composite”, “cell response”, “infection”, “osteomyelitis”, “patterning”, “structuring”, “nanoscale”, and different names of metallic and non-metallic ions.

Ion doping

On the basis of the network former, bioactive glasses can be classified into three categories – silicate, phosphate, and borate – with peculiar features and applications, as recently well reviewed by Baino.³¹ The composition of bioactive glasses



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especially for bone TE, and additionally provide an overview of their effects on bacterial cells, which are often an obstacle to tissue regeneration by causing severe infections.

Doping is defined as the incorporation of a low concentration of an element (ranging from a few ppm to a few percent) into the original material. There are different strategies to insert ions into the glass network, *i.e.* mixing with the raw materials and melting,³¹ ionic exchange^{31,36,37} on melt-derived BGs, as well as the sol-gel technique, which is very versatile and allows the facile incorporation of many ions in the glass composition.^{38–41}

The released ions act as enzyme co-factors, either as coenzymes or prosthetic groups, influencing signaling pathways and stimulating metabolic effects involved in tissue formation.⁴ Thus, they can induce intracellular and extracellular responses.⁴² However, issues associated with the potential toxicity of these ions have to be taken into account.⁴³

In the past several years a new type of bioactive glass has been developed: mesoporous bioactive glasses (MBGs) that possess a higher surface area and a mesoporous structure,⁴⁴ as pioneered by Yan *et al.*⁴⁵ who developed the first SiO₂-CaO-P₂O₅ MBGs. In addition, these glasses are characterized by a faster degradation rate; hence MBGs doped with metallic ions can release their therapeutic or antibacterial ions quicker than non-porous glasses⁴⁶ and show higher bioactivity.^{34,45,47,48} MBGs can be used to fabricate very promising TE scaffolds whose physicochemical and biological properties can be further improved or modified by the incorporation of additional doping ions such as copper (Cu),⁴⁹ lithium (Li),⁵⁰ strontium (Sr),^{51,52} or zirconium (Zr).⁵³

The first bioactive glass that was developed (by Hench and co-workers in the 1970s) is the 45S5 Bioglass®, a Na_2O - CaO - SiO_2 -based glass, but modified with a small amount of P_2O_5 , allowing the synthesis of a chemically more labile material with enhanced bone-bonding ability.^{54,56} The dissolution products of this glass upregulate the gene expression that controls osteogenesis and the production of related growth factors.^{42,57}

Indeed, the release of critical concentrations of soluble silicon (Si) (presumably in the form of $\text{Si}(\text{OH})_4$), calcium (Ca), phosphorus (P), and sodium (Na) ions strongly affect the stimulation of osteogenesis and bone metabolism,⁵⁸ inducing the activation of a synchronized sequence of genes in osteoblasts, that undergo cell division and synthesize an extra-cellular matrix, which mineralizes to become bone,³ as shown by *in vitro*⁵⁹ and *in vivo* results.⁶⁰ Moreover, when implanted into rabbit femurs, micrometric granules of 45S5 Bioglass® were found to promote more rapid bone growth than granules of synthetic HA.⁶¹ The first investigation about the molecular interactions of the ionic dissolution products of 45S5 Bioglass® and their physiological environment was done in 2001 by Xynos *et al.*,⁵⁷ who observed for the first time that a solution containing Si, Ca, and P ions can stimulate gene transcription in human osteoblasts (including genes that (a) encode products that can induce osteoblast proliferation *e.g.*, RCL, (b) participate in the dynamic processes of ECM remodeling *e.g.*, metalloproteinases, (c) perform differentiated functions *e.g.*, CD44, and (d) promote cell-cell and cell-matrix attachment), and induce the secretion of GFs, such as insulin-like growth factor II (a key regulator of osteoblast homeostasis), and vascular endothelial growth factor (which belongs to the fibroblast growth factor family and has osteogenic potential).

To further enhance its properties, 45S5 Bioglass® has been modified by several research groups, incorporating several different doping ions.^{62–66} It was observed that substitutions of 5–15 wt% B₂O₃ for SiO₂ or 12.5 wt% CaF₂ for CaO or inducing crystallization to form glass-ceramics have little influence on the bone-bonding ability of 45S5 Bioglass®, whereas the incorporation of small amounts (3 wt%) of Al₂O₃ prevents bonding to bone.³ More details about how ions released from 45S5 Bioglass® influence the cell cycle are reported in an interesting review by Hench *et al.*¹

Regarding the sodium release from 45S5 Bioglass® and similar glasses, under certain circumstances, this is very fast (initial burst release) and can cause a strong increase in pH, provoking an unwanted cytotoxic effect of these BGs, such as cell death at least under *in vitro* conditions. Thus, as a potential alternative, a new sodium-reduced fluoride-containing BG belonging to the CaO–MgO–SiO₂ system, namely BG1d-BG (with composition in wt%: 46.1 SiO₂, 28.7 CaO, 8.8 MgO, 6.2 P₂O₅, 5.7 CaF₂, 4.5 Na₂O), has been synthesized and already been evaluated *in vitro*, *in vivo* and in preliminary clinical trials.⁶⁷

of large fractures of rat bone was, instead, achieved by Wei *et al.* using porous scaffolds doped with strontium.⁷⁷ Comparable results about the excellent bone regeneration ability of Sr-incorporating BGs were obtained by Zhao *et al.* who used mesoporous scaffolds composed of Sr-doped BGs (Sr-MBG scaffolds) to repair critical-sized rat calvarial defects,⁵² after previous excellent *in vitro* results with MC3T3-E1 osteoblast cells (higher proliferation rates, ALP activity, and expression of osteogenic markers, type I collagen formation and bone nodule formation in comparison with Sr-free scaffolds).⁷⁸ For further information on Sr-doped silicate, borate, and phosphate glasses (including the synthesis methods, structure, reactivity and applications), a review by Kargozar *et al.* is available since 2019.⁸¹

Being the second most abundant mineral element in the human body and along with calcium a key component of the mineralized matrix, phosphorus plays many important roles in numerous body processes and the release of phosphate groups (such as orthophosphate PO_4^{3-} , linear species such as pyrophosphate ($\text{P}_2\text{O}_7^{4-}$) and tripolyphosphate ($\text{P}_3\text{O}_{10}^{5-}$) and cyclic species like cyclic trimetaphosphate $\text{P}_3\text{O}_9^{3-}$), from BGs, can enhance tissue regeneration.⁵⁴ The addition of 10 mmol inorganic phosphorus in a cell culture medium has been shown to stimulate the expression of key regulator agents for bone tissue formation, the matrix Gla protein (MGP), in osteoblastic cells.⁸² Phosphate-based glasses are an ideal medium for the controlled release of therapeutic ions, because the weight loss and the related ion release are generally sustained over time and highly linear for a wide range of glass compositions and types, dissolving in a congruent and uniform way (due to acid- or base-catalyzed hydration of the polymeric phosphate network), differently from silicate glasses that dissolve mainly in an incongruent manner.⁵⁴ In any case, P could be not necessary for *in vitro* ECM mineralization, because even phosphate-free silicate glasses, such as the sol-gel derived 70S30C (mol%: 70SiO₂-30CaO) synthesized by Jones *et al.*,⁸³ have been demonstrated to enhance osteoblasts to mature, differentiate and produce bone-like minerals. Anyway, the authors of the actual review agree with Hoppe *et al.*⁴ that these last reported results should not underestimate the importance of phosphate glasses as therapeutic ion carriers and bone metabolism regulating agents.

Among others, the skeleton contains a significantly large amount of zinc, which plays an essential role in the formation and mineralization of bone,⁵⁴ as indicated by its accumulation in the growth plate and its concentration in a line along cancellous and cortical bone calcifying fronts. Remarkable for bone mineralization is also the fact that zinc is a key element in ALP, an enzyme that is central to the mineralization of bone matrix.⁸⁴ The importance of zinc for bone tissue formation is also confirmed by the negative effects of zinc deficiency, which is demonstrated to be associated with a retardation of skeletal growth and alterations in bone calcification.⁸⁵ In contrast, positive effects on bone metabolism have been obtained through dietary supplementation with zinc.⁸⁶ Moreover, the activities of matrix metalloproteinases that remodel the collagenous ECM of bone are dependent on zinc. Thus, it is clear

that zinc can be potentially used as a bone regeneration therapeutic agent, as widely discussed in a recent review by O'Connor *et al.*⁸⁷ about the role of zinc in the growth, homeostasis, and regeneration of bone. Various BGs⁸⁸⁻⁹¹ for bone TE have been doped with zinc, taking advantage of its ability to influence the osteogenic differentiation of human bone marrow-derived mesenchymal stromal cells (hBMSC) and the osteoclastic differentiation of monocyte/macrophages *in vitro*, and to encourage the attachment and proliferation of osteoblasts, and to inhibit osteoclastic cells.⁹²

Also, magnesium is very important in the bone matrix (which, in fact, contains 0.72 wt% of Mg⁹³) and has stimulatory effects on the development, mineralization, and maintenance of bone.³⁸ It has been shown that the addition of magnesium in bioactive glasses is favorable for human osteoblast-like cell proliferation and function.⁹⁴ A review of the effect of Mg ions on glass properties and bone cell response (*in vitro* and *in vivo*) was published in 2012 by Diba.⁹³ Detailed information about glass compositions, Mg content (wt%), and the results of *in vitro* biological tests are reported in Table III of that review.⁹³ The applications of Mg-doped BGs in scaffolds, bone cements, and bioactive coatings are also reviewed.⁹³ The *in vivo* osteogenic ability of Mg-doped BGs (with composition 45SiO₂-3P₂O₅-26CaO-15Na₂O-7MgO-4K₂O, mol%) has been further confirmed by implanting the BG-derived scaffolds into surgically created critical-size bone defects in rats, as published by Kargozar *et al.* in 2022.⁹⁵

However, even ions not naturally contained in bone can positively affect bone tissue regeneration.

Cerium (Ce) was proved by Zhang *et al.*⁹⁶ to display a positive effect on the proliferation, differentiation, and mineralization of primary osteoblasts when cultured in a medium containing Ce³⁺ ions. Indeed, all tested concentrations (1×10^{-9} , 1×10^{-8} , 1×10^{-7} , 1×10^{-6} , 1×10^{-5} , and 1×10^{-4} mol L⁻¹) of Ce³⁺ promoted the proliferation of primary osteoblasts whereas their differentiation, adipocytic transdifferentiation and mineralization depended on the cerium concentration and on the culture time, with – for example – the differentiation promoted on the first and third day at concentrations of 1×10^{-9} , 1×10^{-7} , and 1×10^{-6} mol L⁻¹, but inhibited at higher concentrations.⁹⁶ The pro-osteogenic properties of Ce ions were also confirmed by Zheng *et al.*,⁹⁷ who observed the expression of osteogenesis-related genes in osteoblast-like Saos-2 cells cultured with Ce-containing BG particles.

Copper is mostly known for its antibacterial properties, but the reduction of osteogenesis and the lowered mechanical properties of bones in case of either dietary or genetic copper deficiency in both humans and animals are clues that copper could possess also interesting osteogenic properties. Copper is, in fact, essential for several enzyme-based processes for bone formation and bone maintenance. Anyway, this does not explain why the localized or systemic delivery of copper should be beneficial for the healing and correction of copper deficiency, but at the same time, it is not possible to deny this hypothesis without any opposite evidence, whereas results showing the stimulation of proliferation and differentiation of



MSCs cultured in osteogenic differentiation medium containing a Cu concentration around 0.1 mM have already been published.⁹⁸ These results have been confirmed by more recent results of Westhauser *et al.* who have cultured mouse bone marrow-derived mesenchymal stromal cells (BMSCs) in media containing the ion dissolution products of 70SiO₂-25CaO-5X where X is CuO or MnO or ZnO (mol%),⁹¹ and Rau *et al.* who have proved that the Cu-doping of 45S5 Bioglass® (with a composition in wt% of 45SiO₂-24.5Na₂O-6P₂O₅-19.5CaO-5CuO) is effective in stimulating the early differentiation of MSCs to the osteoblast phenotype, through the over-expression of anti-inflammatory interleukins and the reduction of pro-inflammatory interleukins.⁹⁹ In addition, Lin Y. *et al.* have confirmed previous studies showing that the addition of 0, 0.4, 0.8, and 2.0 wt% CuO in silicate 13-93BG scaffolds (with a composition in wt% of 53SiO₂-6Na₂O-12K₂O-5MgO-20CaO-4P₂O₅) enhance the migration and osteogenic differentiation of BMSCs.¹⁰⁰ However, it has been demonstrated that at high concentrations copper may inhibit osteogenesis *via* the down-regulation of osteogenic genes such as *RUNX2*, which is the main regulator of osteoblast phenotype, as well explained in the review by Kargozar *et al.*,¹⁰¹ which contains an interesting overview of the effects and applications of Cu-containing BGs and bioactive glass-ceramics, with detailed information about glass compositions and dopant concentrations; therefore, the actual review will not provide further details.

Boron (B) interacts with the performance of several metabolic enzymes and its osteogenic properties are well known, as broadly reviewed by Balasubramanian *et al.*⁵⁸ and confirmed by various *in vitro*¹⁰² and *in vivo* studies.¹⁰³⁻¹⁰⁷ Indeed, impaired bone growth and abnormal bone development can be associated with boron deficiency, both in humans and animals.⁵⁸ Optimal levels of boron are, in fact, necessary for calcium metabolism, which is strongly related to the bone tissue metabolism process.⁵⁸ Different compositions of BGs containing boron, including boron-doped, borosilicate, and borate glasses, have been synthesized as promising suitable carriers of boron for bone TE applications.^{58,108} Recently, it was shown that the incorporation of boron into BG scaffolds leads to a controllable release of boron ions with a significant improvement in the proliferation and bone-related gene expression (*Col I* and *RUNX2*) of osteoblasts.¹⁰⁹ Recently it has been shown that certain compositions of borate BGs enhance osteogenesis to a greater extent than 45S5 Bioglass®, although these borate glasses contain no Si.² However, a concern associated with the toxicity of borate ions, (BO₃)₃, released from borate has been recently raised, because some borate BGs have been demonstrated to be toxic to cells in *in vitro* cultures (especially in conventional “static” conditions, whereas in “dynamic” culture conditions the toxicity was diminished).²

The effect of fluorine on the regeneration of hard tissues such as bone and teeth is controversial. Concentrations of 25–500 ng ml⁻¹ of fluoride ions have been demonstrated to stimulate osteoblast cells whereas concentrations of more than 500 ng ml⁻¹ can suppress osteoblast activity.¹¹⁰ In fact, high fluoride doses can be toxic for cells, causing oxidative cell

damage¹¹¹ and leading to dental and skeletal fluorosis.¹¹² Alkasite, an alkaline calcium fluorosilicate BG without phosphate, based on the system SiO₂-CaO-Na₂O-CaF₂, can be used as an additive in resin composites for dentistry applications. Clinical trials confirmed that it offers better mechanical, aesthetic, and technical performance in comparison with resin-modified glass ionomer cements.¹¹³

For a decade, lithium has been suggested as a novel therapeutic ion for bone TE. In 2011 Khorami *et al.*⁶² synthesized different lithium-containing Bioglass®-based glasses, by substituting 0–12 wt% Li₂O for Na₂O. They found that the proliferation rate and ALP activity of newborn rat calvaria-derived osteoblastic cells cultured on Li-substituted glasses were higher than those of Li-free glasses in a dose-dependent manner. Many other experimental results studies are now available, as well as reported by Durand *et al.*¹¹⁴

Manganese has been shown to support the proliferation of osteoblastic cells.^{91,115} In 2014 Miola *et al.*¹¹⁵ reported the synthesis of new BGs belonging to the system SiO₂-P₂O₅-CaO-MgO-Na₂O-K₂O doped with different amounts of manganese oxide (MnO) and their good osteogenic properties. Cellular tests performed on these Mn-doped BGs, up to a concentration of 50 µg cm⁻² (µg of glass powders/cm² of cell monolayer), showed that the presence of Mn was not cytotoxic for MG-63 osteoblasts (cultured for up to 5 days with the synthesized Mn-doped glasses) and allowed a good proliferation, differentiation and spreading of osteoblasts on Mn-doped glasses, as shown by the ALP increase, expression of some bone morphogenetic proteins (BMPs), and calcium deposit.¹¹⁵ However, in some compositions at high concentrations, Mn can have cytotoxic effects on bone MSCs.⁹¹

Niobium (Nb) is very biocompatible and has been shown to reduce the cytocompatibility of biomaterials.¹¹⁶ It is also associated with an increase in ALP activity and enhanced calcification in bone defects.¹¹⁷ Therefore, the incorporation of Nb in bioceramics is of great interest. Among the several incorporation methods, the sol-gel synthesis is the most promising in the case of niobium addition, because the adopted lower processing temperatures avoid the formation of strong bonding as in the case of the melting process, thus not causing a reduction in glass solubility and degradation, as shown by de Souza Balbinot *et al.*¹¹⁶ who have synthesized sol-gel Nb-doped BG powders and scaffolds. An increase in cell mineralization related to the formation of a higher area fraction of mineralized nodule was observed after 14 days in the case of Nb-doped BG powders (in 5 mg concentration) when compared with BAG (*p* < 0.05).¹¹⁶ The synthesized Nb-doped scaffolds presented an even higher mineralized area when compared with Nb-doped powders, probably because of increased interface interactions related to macroporosity,¹¹⁸ and an increase in cell mineralization already after 7 days in comparison with undoped scaffolds, whereas no statistical difference was found in the cell proliferation rates between powders and scaffolds and in the case of the addition of niobium,¹¹⁶ as instead found by Pradhan *et al.*¹¹⁹

45S5 Bioglass® doped with titanium (Ti) synthesized by Vrouwenvelde *et al.* has been shown to exhibit a higher pro-



Bioactive ceramics can also promote angiogenesis (or neo-vascularization), as already reported by Kargozar *et al.*¹²⁷ and here further reviewed. Various ions have been proved to promote angiogenesis, which plays a critical role in tissue regeneration and is highly beneficial in the case of large tissue-engineered

All the here-described anticancer procedures (hyperthermia, photothermal therapy, and local radiotherapy using

radioactive BGs) may be used together with chemotherapy or surgery, taking advantage of the synergistic therapeutic benefits of different antitumoral strategies. In addition, in this context, the regenerative properties of BGs should not be ignored, allowing them to successfully heal bone tumor defects.

For further information about the use of BGs and bioactive glass-ceramics in cancer therapy, the reader can refer to a review of Moeini, published in 2023.¹⁴⁴

Bioactive properties

The ion exchange between the ions released from the glass matrix and the body fluids (or SBF solution, in the case of *in vitro* acellular bioactivity tests) can influence the bioactivity performance and HA deposition rate.

For example, a low amount of small fluoride ions seems to enhance the formation of apatite (in detail, fluorapatite) of higher crystallinity, even at low pH conditions (below 6), but more fluorine is not necessarily beneficial for apatite formation, leading to the formation of fluorite at the expense of apatite by increasing the fluoride content in the glass.¹⁴⁶ This ability of fluoride ions to increase glass bioactivity was also confirmed by studies on F-doped phosphate glasses.¹⁴⁷ Moreover, as fluoride is a small ion, if substituted with bigger ions, it allows the formation of a very ordered, highly crystalline apatite. Indeed, the formed HA is highly influenced by the size of the substituted molecules.

In contrast to fluoride ions, bigger carbonate ions, which substitute into two sites, tend to form a more disordered amorphous HA.¹⁴⁸

As expected, since calcium is a main component of natural HA, a proper amount of calcium inside the BG network is essential for the ion exchange between glass and biological fluids, that is responsible for the HA deposition on the BGs,³¹ and Ca-doped glasses showed an increased calcium deposition in *in vitro* cell cultures by increasing the release of Ca²⁺ ions into the culture medium.⁷⁴

Other studies show an increase in glass bioactivity and bone mineralization in the presence of manganese in the BG,¹¹⁵ although other studies reported that Mg has no effect or even a negative effect on apatite formation, not inhibiting, but retarding its deposition.¹⁴⁹

It seems to be confirmed that bioactivity can be increased by incorporating boron ions in the bioactive glass, partially replacing the SiO₂ in silicate glasses with B₂O₃,^{58,150} and the total substitution of silicon with boron has resulted in higher bioactive borate glasses, characterized by low chemical durability and a fast HA conversion rate.^{151,152} It is interesting to notice that the bioactivity of borate BGs can be further increased by incorporating in the glass network fluoride ions, which render the glass network itself more soluble, as shown by ElBatal *et al.*, who compare the bioactivity of Na₂O–CaO–B₂O₃ and NaF–CaF₂–B₂O₃ glasses,¹⁵³ or strontium ions, as reported by Marzouk *et al.*¹⁵⁴ who observed an increase in the glass corrosion rate by increasing the SrO content, or even

vanadium ions, which increase the degradation rate of the borate glasses.¹⁵⁵

However, if borate glasses are doped with copper, their HA precipitation rate decreases,¹⁵⁶ but in general, the influence of copper on glass bioactivity is still under investigation and very argued. Most experimental works have shown a bioactivity reduction in the case of doping with copper, but there are even experimental results showing that Cu-doping does not alter the glass bioactivity; *e.g.* the bioactive glasses with a superficial *in situ* chemical and physical reduction of copper fabricated by Miola *et al.* maintained their bioactivity up to 14 days (the duration of the performed acellular bioactivity test),¹³⁴ or even accelerate apatite formation in case of Cu-doped BG nanoparticles containing 10–15 wt% Cu (10Cu-BGN and 15Cu-BGN) in comparison with 5Cu-BGN and undoped BG nanoparticles.¹⁵⁷

Not only the effect of copper, but also the effect of magnesium on glass bioactivity is still unclear. Indeed, past studies showed that Mg did not inhibit the apatite-forming ability of BGs but Mg was believed to retard the time of apatite deposition, because of the negative effect of MgO that suppressed calcium release and thus hindered silica-gel layer formation,^{93,158} although new results (published in 2020¹⁵⁹) on sol-gel Mg-doped SiO₂–CaO–P₂O₅ have shown that the amount of apatite crystals increased in samples doped with 8 mol% of magnesium, whereas a lower Mg content was not effective in increasing glass bioactivity. These results have been also confirmed by recent studies (published in 2020¹⁶⁰) on innovative magnesium–lanthanum dual-doped BGs, showing an increase in apatite formation and glass bioactivity in all five synthesized glass compositions (1 wt% lanthanum with 0, 1, 2.5, 5, and 10 wt% magnesium), especially in the BGs with the higher Mg content.

Gallium, instead, has been shown to delay the growth of a crystalline apatitic layer on Ga-modified glasses with respect to the Ga-free glass, probably because of the dramatic changes induced, at the glass/SBF interface, by the presence of the Ga₂O₃ component,¹⁶¹ although gallium protects the formed HA matrix, thereby improving the biomechanical properties of the newly formed bone and in general of the skeletal system.³⁴

Besides gallium, also cerium decreases HA formation,⁶⁵ and the higher its concentration, the lower the quantity of formed hydroxyapatite because the presence of these dopant ions leads to the formation of CePO₄ at the expense of HA.⁶⁵

Comparing a SiO₂–CaO–P₂O₅ glass with SiO₂–CaO–P₂O₅ glasses modified with cerium, gallium, or zinc ions, Salinas *et al.* observed that zinc-doped BG shows the lowest bioactivity.³⁴

Similar results were found in the case of 45S5 Bioglass®⁶³ and borate glasses⁵⁸ doped with zinc, confirming that Zn ions are inhibitors of HA crystal formation,¹⁶² because of the ability of ZnO to act as an intermediate oxide (*i.e.* to act either as a network former ZnO₄ or as a network modifier like alkali oxides and alkaline earth oxides), forming tetrahedral species ZnO₄^{2–},^{92,163} especially in the case of very high zinc concentration (above 20 wt%), that can cause a significant reduction



in dissolution behavior, leading to the incapability of the glass to form HA (as reported by Aina *et al.*¹⁶⁴), although a few opposite results show an increase in glass bioactivity when a small amount of zinc is added to the glass composition. Abdelghany *et al.*⁹² have shown that increasing the ZnO amount (from 2 mol% to 10 mol%) in soda lime borate glasses favors HA formation. Balamurugan *et al.*¹⁶⁵ have demonstrated that the incorporation of 5 mol% of zinc into a sol-gel CaO-P₂O₅-SiO₂-ZnO system did not diminish the bioactivity and Salinas *et al.*³⁴ have observed a rapid *in vitro* bioactive response when the amount of ZnO is less than 4 mol%. However, in contrast, according to Shahrabi *et al.*,¹⁶⁶ the addition of 5 mol% of ZnO can cause the reduction of the number of non-bridging oxygen atoms, and so leads to a decreased bioactivity. Zn- and Ag-co-doped nano-sized glass particles³² showed an initial increase of crystallized apatite deposition, followed by a delay in the HA formation, which was more pronounced in the case of the glasses incorporating zinc ions, in comparison with undoped glasses used as a control. In addition, MBGs are characterized by an extraordinary bioactivity which can be prevented anyway if zinc is added to the composition of a highly bioactive mesoporous glass, as recently reported by Nešćáková *et al.*,⁹⁰ showing that the glass composition affects the glass bioactivity more than its surface area.

However, when Zn and Fe are added together, by increasing the combined Zn-Fe content, their combined effect leads to an increase in the glass bioactivity.¹⁶⁷ An enhancement in the growth of the apatite layer was also observed in the sol-gel BGs of an $x\text{ZnO}(22.4 - x)\text{Na}_2\text{O}-46.1\text{SiO}_2-26.9\text{CaO}-2-6\text{P}_2\text{O}_5-2\text{MgO}$ system, probably due to the co-presence of magnesium ions and zinc ions.¹⁶⁸

Another ion that has been shown to inhibit bioactivity is cobalt.⁶⁶ The presence of peaks attributable to HA was, indeed, not observed in the X-ray diffraction (XRD) patterns of the Co-doped BGs (BG.4Co) and B- and Co-co-doped BGs in the case of a higher concentration of doping cobalt ions (BG.2B4Co), whereas the XRD patterns of B- and Co-co-doped BGs in the case of a higher concentration of doping boron ions (BG.10B4Co) were characterized by the presence of the typical diffraction lines of hydroxy carbonated apatite (HCA), confirming the ability of boron to stimulate bioactivity.⁶⁶ However, opposite results were obtained in the case of BGs belonging to the Na₂O-CaO-SiO₂-P₂O₅ glass system by Vyas *et al.* In this study, bioactivity and mechanical behavior were enhanced by adding cobalt ions to the glass composition.¹⁶⁹ The *in vitro* apatite-forming ability of these Co-doped BGs was significantly improved by increasing Co amounts.¹⁶⁹ In the case of a moderate (2.5 mol%) incorporated amount of CoO, the effects on glass dissolution and apatite-forming ability became almost negligible, without a significant difference in comparison with the Co-free sol-gel BGs.¹⁷⁰ Further information about the dissolution rates and *in vitro* bioactivity of Co-doped BGs can be found in the already-mentioned review of Baino *et al.*²²

It is interesting to point out that the incorporation of strontium can lead to a decrease in the glass dissolution and consequently in the kinetics of the ion release, causing a delayed

bioactivity, because strontium has a large atomic radius that likely blocks the movement and release of other ions.^{39,58,78,171} When Sr ions are substituted with smaller ions in the glass composition of sol-gel BG particles, the obtained BG particles are characterized by a higher surface area and lowered bioactivity, as demonstrated by Hoppe *et al.*,¹⁷² who synthesized Sr-doped 1993 glass nanoparticles by substituting various amounts of CaO with SrO. However, when strontium ions are added to sol-gel-derived zirconium-doped BGs, leading to a glass ZS-BGs containing 60% of SiO₂, (36 - X)% of CaO, 4% of P₂O₅, 5% of ZrO₂ and X% of SrO (where X = 0, 3, 6, 9, and 12 mol%), the glass bioactivity is increased.¹⁷³ Indeed, the addition of Zr strengthens the connectivity of the glass network, reducing the release rate of Si, P, and Ca ions during the acellular bioactivity test, but in contrast, the addition of strontium has a weakening effect, which is stronger than the strengthening effect of zirconium.¹⁷³

Surprisingly, even phosphorus could potentially hinder the bioactivity, because of the higher affinity of modifier Na and Ca cations for coordinating phosphate rather than silicate, leading (in combination with the formation of P-O-Si linkages) to an increase in the repolymerization of the silicate network with increasing P₂O₅ content, but practically this negative effect of P ions is counterbalanced by the increasing amount and related fast release of free orthophosphate groups. Indeed, increasing the content of phosphates has been shown to stimulate *in vitro* HCA deposition.¹⁷⁴ In this same study,¹⁷⁴ increasing amounts of CaO and SiO₂ inhibit the deposition of HCA during immersion in SBF.

Novel results on borate-substituted 45S5 Bioglass® with a basic composition (mol%) of 46.1B₂O₃-26.9CaO-24.4Na₂O-2.6P₂O₅¹⁷⁵ have shown that the sodium content has very little effect on the rate of conversion to HCA in SBF, because even with decreasing the amount of sodium, while maintaining the ratio of the other elements (until the synthesis of a sodium-free glass with a composition in mol% of 61.0B₂O₃-35.6CaO-3.4P₂O₅), the formation of an HCA layer was observed within 2 h from immersion in SBF.

Finally, to provide a more complete possible analysis of ion effect on glass bioactivity, it should be also reported that small additions of Al₂O₃, Ta₂O₅, TiO₂, Sb₂O₃, or ZrO₂ in BG compositions have been shown to induce an inhibition of bone bonding.³ Alumina and zirconia are, usually, added to the bioceramics composition to enhance the structural stability, the mechanical properties, and the corrosion resistance of the bioceramics, by eliminating some of the non-bridging oxygens and hence enhancing the network compactness.^{38,176} Thus, it is remarkable that zirconium does not hinder the bioactivity of the bioceramics.¹⁷⁶ On the other hand, Al₂O₃ addition often significantly reduces the bioactivity, hindering the HCA formation. The concentration of alumina required to suppress the bioactivity depends on the composition or chemical durability of the glass. For example, if Al₂O₃ is added up to about 1.5 wt% in the case of modified 45S5 Bioglass® and 1.5 mol% in case of the CaO-SiO₂-Al₂O₃ system, no significant lowering of the bioactivity can be observed, whereas if CaO-SiO₂-Al₂O₃



In 2016, Miola *et al.* developed an innovative scaffold fabrication method based on a modified polymeric sponge replication technique, doping bioactive glass-ceramic scaffolds with silver directly during the scaffold synthesis.¹⁹⁰ These new synthesized Ag-doped scaffolds were demonstrated to be able to create a significant bacterial inhibition halo of about 2 ± 0.5 mm and their silver release kinetics were considered helpful to treat both the development of infections due to bacterial contamination (direct or indirect) of an implant throughout the surgery and the development of latent infections.¹⁹⁰ It is important to point out that, besides its high effective antibacterial properties, silver does not alter the mechanism and kinetics of glass ceramic bioactivity,¹⁹⁰ as confirmed by other recent studies on Ag-doped borate BGs.²¹² However, it has been shown that glass containing 2 wt% of silver is toxic also to mammalian cells (whereas glasses containing 0.75 and 1 wt% silver are not toxic).³⁸

The antibacterial and antimicrobial capability of silver has been compared with the effect of other ions. For example, nano-sized glass particles with a chemical composition of $60\text{SiO}_2-4\text{P}_2\text{O}_5-(x)\text{Ag}_2\text{O}-(31-x)\text{CaO}$ and $60\text{SiO}_2-4\text{P}_2\text{O}_5-(x)\text{ZnO}-(31-x)\text{CaO}$, where $x = 2$ or 4 , were produced through a sol-gel synthesis by Shahrbabak *et al.*³² An increase in the antibacterial effect of the glasses was recorded by increasing the Zn and Ag content, especially in the case of Ag-containing BGs, which have been shown to be more antibacterial than Zn-containing BGs. The glass containing 4% of silver (BA4 sample) showed the best antibacterial behavior. The pH rise and the ionic concentrations of species released by the glasses under investigation were monitored to determine whether differential changes in these factors between the different BGs could contribute to the antibacterial properties of AgBG, but the changes induced in pH and ionic content were similar to those provoked by AgBG and, therefore, the antibacterial effect of AgBG was attributed only to the released ionic silver.¹⁸⁸

Another well-known antibacterial ion is copper. Among others, in 2005 Abou Neel *et al.* substituted calcium with variable copper amounts in the glass system $\text{P}_2\text{O}_5\text{-CaO-Na}_2\text{O}$, producing BG fibers with compositions (mol%) $50\text{P}_2\text{O}_5\text{-30CaO-20Na}_2\text{O}$, $50\text{P}_2\text{O}_5\text{-30CaO-19Na}_2\text{O-1CuO}$, $50\text{P}_2\text{O}_5\text{-30CaO-15Na}_2\text{O-5CuO}$ and $50\text{P}_2\text{O}_5\text{-30CaO-10Na}_2\text{O-10CuO}$.¹⁹⁵ Their results showed that the addition of CuO into the glass fibers was effective in reducing the number of bacteria attached to the fibers, in a Cu^{2+} dose-dependent manner, with higher antibacterial efficacy in the case of the higher CuO amount. Indeed, 10%Cu-doped BG was able to deliver a higher concentration of Cu^{2+} , preventing better bacterial colonization and reducing the number of viable bacteria in the local environment, an effect which may be due to either the reduction of bacteria in solution or the reduction in the viable bacteria attached to the fibers. In 2019, Miola *et al.* showed that a copper-doped glass through an ion-exchange technique (SBA3) was able to produce an inhibition zone of about 2–2.5 mm, 2 mm, and $1.5 \text{ mm} \pm 0.5 \text{ mm}$, respectively, as a consequence of the presence of free Cu^{++} ions in the glass network, alone or in synergy with Cu^0 nanoparticles.¹³⁴

In 2020 the excellent antibacterial properties of Zr-doped sol-gel silicate BGs (in particular the one with a composition, in mol%, of $60\% \text{SiO}_2\text{-31CaO-4P}_2\text{O}_5\text{-5ZrO}_2$) have been reported, showing that the addition of zirconium enhances the antibacterial activity of BGs.¹⁷³

Moreover, some of the abovementioned ions, such as boron, cobalt, gallium, lithium, and silver, possess not only antibacterial properties, but also antiviral abilities.¹⁴⁵

Further details about antibacterial and antiviral ions (in detail, Ag, B, Bi, Ce, Co, Cu, Fe, Ga, Mn, Li, and Zn) and the concentrations required to obtain these effects can be found in the review published by Kargozar *et al.* in 2020²¹³ and in the one by Awais *et al.* in 2022.¹⁴⁵

The antibacterial potential of doped mesoporous BGs has been recently reviewed by Kargozar *et al.*²¹⁴ and an interesting table recapping the biomolecular mechanisms behind the action of antibacterial ions released from BGs is available in another review.²¹⁵

Antioxidant properties

Bioactive glasses have been also doped with elements with antioxidant behavior, such as cerium,^{97,216} selenium,²¹⁷ and more recently tellurium.²⁰⁷

Cerium has been investigated in different biological applications due to its ability to switch between Ce^{4+} and Ce^{3+} oxidation states and protect cells against the damage resulting from ROS production.²¹⁶

Recently, Miola *et al.*²⁰⁷ investigated the possibility to dope a BG composition with a low amount of tellurium dioxide, to impart antibacterial and antioxidant properties. The authors demonstrated that Te-doped glasses protect the metabolic activity of cells from apoptosis induced by H_2O_2 treatment and at the same time that Te-doped glasses possess antimicrobial activity and preserve the bioactivity and the cytocompatibility.

Loading with drugs and natural extracts

Bioactive glasses and glass-ceramics can be loaded with various drugs, as demonstrated in many experimental studies.^{120,218,219} Differently from ions, drugs cannot be incorporated into the glass during melting synthesis (because biomolecules suffer from thermal degradation in the case of high temperatures such as the ones involved in the melting of glass precursors),²²⁰ but they can be mixed into the sol suspension during low-temperature sol-gel methods that do not need high temperature treatment to stabilize the formed gel, as shown by some experimental studies.²²¹ To enhance drug loading, the use of porous glasses (in particular, mesoporous glasses) is advantageous, as shown by various experimental investigations.^{9,49,168,222,223} Mesoporous BGs offer also the advantage of slow drug release kinetics, which may be related to both the presence of many Si-OH groups in the internal part of MBG mesoporous channels and the hydrogen bonds and van der Waals forces that are formed between the MBG and the loaded drugs.⁹

Another way to prepare drug-releasing BGs, in particular porous BG scaffolds, is to coat them with a drug-loaded poly-



meric solution. For example, Li *et al.* fabricated vancomycin-releasing porous 45S5 Bioglass®-based glass-ceramic scaffolds by immersing the scaffolds into a solution consisting of PHBV and vancomycin.²²⁴

To cite a few examples (recapping the experimental studies of drug-loaded biomaterial mentioned in this review), on the basis of the final application, different drugs are available and have been already successfully loaded in BGs:

- dexamethasone and dimethyloxallyl glycine to enhance osteogenesis;⁹
- ampicillin,¹⁸ ibuprofen,⁹ and vancomycin^{224,225} for conferring antibacterial properties;
- doxorubicin,^{9,120,226} 5-fluorouracil,^{225,227} and mitomycin C²²⁸ for the inhibition of the viability of cancer cells.

In 2018 mesoporous sol-gel 58S nanoparticles were successfully loaded with propolis and cranberry, to impart antibiofilm properties to the BGs for potential bone healing and infection treatment procedures.²²⁹ Information can also be found in the review published by Kaou in 2023.²³⁰

This review will not further enter into details of the used drugs, as a review about BGs as carriers for therapeutic drugs, growth factors, and proteins, for bone TE, has already been published by Hum and Boccaccini in 2012²²⁰ and a rich overview table written by Baino *et al.* is available in their review.⁴⁴

Applications of doped and loaded bioactive glasses

Among the possible applications of doped bioactive glasses and glass-ceramics, we can find the fabrication of multifunctional glass scaffolds,^{41,49,231,232} and the use as fillers in polymeric matrix or bone cement to obtain composite scaffolds able to release therapeutic ions^{6,8,42,163} or composite bone cement with enhanced osteogenic^{233,234} and antibacterial properties,^{235–237} respectively.

BGs releasing drugs with several therapeutic and/or antibacterial effects can be also added to bone cement.²²⁶

The use of BGs as fillers in degradable matrices might offer a promising strategy for the regulated *in situ* secretion/expression of angiogenic growth factors (*e.g.*, VEGF) and osteogenic markers (*e.g.*, ALP) in therapeutic levels, leading to successful vascularization and bone formation of TE scaffolds.⁶ Composite scaffolds composed of bioactive glasses and polymers can be fabricated using different techniques such as electrospinning, 3D printing, salt leaching, and the slurry sponge method (just to cite a few examples), but the detailed description of these fabrication methods is out of the aim of this review. The interested reader can find more information about composite glass-polymer scaffolds in the following publications, for bone, dental,²³⁸ and cartilage TE,²³⁸ respectively, whereas for antibacterial PMMA-based bone cements a recent review by Bistolfi *et al.* is available.²³⁹

Not only solid scaffolds but also composite osteoconductive and antibacterial hydrogels can be fabricated by incorporating GFs and BG particles inside the hydrogels.^{240–242}

Furthermore, composite bone cements incorporating magnetic BGs can be produced for hyperthermia treatment applications as done a few years ago by Miola *et al.*²⁴³

Doped bioactive ceramics are also useful to coat various surfaces and scaffolds, in order to impart multifunctionality (such as bioactivity and/or antibacterial properties) to the coated material, as shown by many experimental works.^{244–250} Because of the well-demonstrated antibacterial property of silver, silver-containing BG powders have been largely used for coating polymeric scaffolds, resulting in a bioactive and bactericidal implant.^{244,248} Many coating techniques are available, but atmospheric plasma spraying is probably the most popular one because of its good mechanical performance (bond strength and mechanical properties) and the excellent preservation of the amorphous structure of the sprayed BG powder.²⁴⁶ For a comprehensive review of glass-based coatings on biomedical implants, see the review published in 2017 by Baino and Verné.²⁵¹

Synergic effects and summary

In Table 1, the properties of doping ions are summarized. It is possible to observe that several ions realize opposite effects on eukaryotic and bacterial cells, promoting bone and blood vessel regeneration, but having an antibacterial behavior, thus inducing bacteria death.

As already reported, several ions possess more than one property. For example, boron, cerium, copper, lithium, and zinc show both osteogenic and antibacterial properties, as demonstrated by numerous experimental studies.^{49,97,134,202} In addition, it should be reminded that boron^{128,129} and copper^{132–134} possess also angiogenic abilities. The multipotential ability of these ions makes them amazingly interesting for applications related to bone TE and bone surgery.

For example, the nano-sized glass particles produced by Shahrabak *et al.*³² (mentioned above in the subsection “Antibacterial and antiviral properties”) were not only antibacterial, but also bioactive and enhanced G292 osteoblastic cell proliferation, being good candidates for bone TE applications.

Recently, in 2020, Zheng *et al.*⁹⁷ reported the synthesis of antioxidant, anti-inflammatory, and pro-osteogenic BGs containing cerium. A comprehensive review of Ce-containing BGs has been published in 2021 by Zambon *et al.*²⁵²

Besides its antibacterial and antioxidant properties, tellurium is well known for its high conductivity and piezoelectricity, a property that can help stimulate bone growth and regeneration. In fact, bone tissue is sensitive to electrical stimuli. For these reasons, new applications of Te-based glasses and glass-ceramics are emerging, not only thermoelectric applications²⁵³ but also biomedical ones.^{207,254}

Similar observations could be made on fluorine for dental surgery. Indeed, since long ago it has been well-known that fluorine can enhance apatite deposition and in recent years its antibacterial ability has been proved by Liu *et al.*¹⁴⁷ who demonstrated that the growth of sub-gingival bacteria (*Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*) was significantly inhibited after incubation with F-doped BG particulates.

However, it should be also pointed out that recently there is an increasing interest in co-doping in tissue engineering,



Table 1 Properties of doping ions

Properties						
Ion	Osteogenic	Angiogenic	Antibacterial/antiviral	Antitumoral	Antioxidant	Bioactive
Ag			Yes ^{32,36,145,188-190,192-194,213,215,244,245,248,255,257}	Yes ^{144,145}		Not modified ^{190,212} or delayed ³² depending on the Ag amount
Al	No, it prevents bone bonding ³					Inhibited ⁵ or no effect if ions are incorporated in crystalline phases ³⁸
B	Yes ^{102-107,109,145,269,276}	Yes ^{66,128,129,145,213}	Yes ^{58,145,277}	Yes ¹⁴⁵		Enhanced ^{150,153}
Ba			Yes ¹⁸⁶			Not compromised ¹⁸⁴ but diminished if co-doping with Fe ¹⁸⁶
Br			Yes ²⁰⁰			Enhanced ⁷⁴
Ca	Yes ^{4,72,73}	Yes ^{130,213}		Yes ¹⁴⁴	Yes ^{97,216,252}	Lowered ^{65,216}
Ce	Yes ^{96,97,252}	Yes ^{71,31,252}	Yes ^{97,198,213,215,252,257}			Argued influence: enhanced ¹⁶⁹ or inhibited ⁶⁶
Co	Yes ¹²⁶	Yes ^{66,76,126,129,132,145,213}	Yes ^{145,201}	Yes ¹⁴⁴		Argued influence: enhanced ¹⁵⁷ or lowered ¹⁵⁶ or even no effect ¹³⁴
Cu	Yes ^{49,91,98}	Yes ^{49,64,132-134,213,215,274,278}	Yes ^{49,133,134,140,145,195,213,215}	Yes ^{144,145}		Enhanced ^{146,147,153}
F	Yes ¹¹⁰		Yes ¹⁴⁷			Enhanced if combined with zinc ¹⁶⁷
Fe	Yes ⁹		Yes ^{140,145}	Yes ^{41,141,144,243}		Delayed ¹⁶¹
Ga	Yes ⁹	No ¹³¹	Yes ^{161,196-198,213,215}	Yes ¹⁴⁴		Enhanced ¹⁷⁷
Ge						Enhanced ¹⁸¹
Gd	Yes ¹⁸⁶					Enhanced ^{160,186}
La	Yes ¹⁸⁶	Yes ¹⁸⁶				
Li	Yes ^{62,114,202}	Yes ¹³⁵	Yes ^{145,192,202}			
Mg	Yes ^{94,95,145}	Yes ^{136,137,213}				Argued influence: enhanced ^{159,160,168} or not compromised ⁹⁵ or delayed ¹⁴⁹
Mn	Yes ^{91,115,186}		Yes ^{145,186,204}	Yes ¹⁴⁴		Argued influence: enhanced ^{115,186} or delayed ¹⁴⁹
Na						Little influence ¹⁷⁵
Nb	Yes ^{116,117,119,186}	Yes ¹⁸⁶		Yes ²²⁶		Enhanced if sol gel-sol BG, ^{116,117,178} whereas reduced if BG produced by melting-quenching ^{179,180}
P	Yes ^{54,82}	213				Enhanced ^{174,279}
Rb	Yes ^{186,205,280}	Yes ^{186,205}	Yes ²⁰⁵	Yes ²⁰⁵		Enhanced ^{182,186}
Se	Yes ¹²⁵		Yes ^{125,186,217,281}	Yes ^{123,144}	Yes ²¹⁷	Good ^{120,281}
Si	Yes, ^{68,71,138} however sometimes argued ⁷⁰	213				
Sb						Inhibited ³
Sr	Yes ^{39,51,52,74,76-80,106,138,145,171,233,245,269}	Yes ^{52,76,138,282}	Yes ^{39,78,281}	Yes ²⁸¹		Argued influence: enhanced ^{58,154,173,249,255} or lowered ¹⁷² or delayed ^{39,58,78,171}
Ta			Yes ^{186,206}	Yes ²⁰⁶		Argued influence: enhanced ¹⁸⁶ or inhibited ^{3,186} depending on the added amount
Tb					Yes ²⁰⁷	Enhanced ^{183,186}
Te			Yes ²⁰⁷			Inhibited ³
Ti	Yes ⁴		Yes ^{208,209}			Enhanced ¹⁵⁵
Va		No ¹³¹				





Table 1 (Contd.)

Properties		Antibacterial/antiviral	Antitumoral	Antioxidant	Bioactive
Ion	Osteogenic				
Yb	Yes ^{62,86,88,89,91,92}	Yes ^{32,137,145,199,203,213,215,268}	Yes ^{144,256,283}		Enhanced ^{181,186} Argued influence: delayed ^{32,34,58,63,166,264} or even inhibited ^{3,34,90,92,162,163,247} or in contrast not diminished ¹⁶⁵ or enhanced (depending on different amounts and co-doping with Mg or Fe) ^{34,92,167,168} Argued influence: inhibited ³ or diminished ¹⁸⁶ or in contrast not hindered ³³ or even enhanced, ¹⁸⁶ especially in synergy with Sr addition ¹⁷⁶
Zn					
Zr	Yes ^{9,53,173,186}	Yes ^{173,186}			

which allows multifunctionality to be reached by taking advantage of the different properties or different mechanisms of functioning of different doping ions. For example, co-doping with Ag and Li,¹⁹² Ag and Sr,²⁵⁵ Ag and Zn,^{32,256} Ag and Ce,²⁵⁷ B and Co,^{66,129} B and Cu,^{108,258,259} B and Zn,²⁶⁰ Cu and Co,²⁶¹ Cu and Fe,²⁶² Cu and Sr,²⁶³ Cu and Zn,^{215,258,261,264} Si and Sr,¹³⁸ Sr and Co,⁷⁴ Sr and Li,²⁶⁵ Sr and Zn,²⁶⁶ Zn and Co,²⁶⁷ and Zn and Mn²⁶⁸ has been studied.

For example, Anand *et al.* fabricated multifunctional BGs by introducing amounts up to 2 mol% of Sr and Cu ions as co-dopants in the ratio 1 : 1 in MBGs and demonstrated that such novel BGs could be beneficial for bone tissue regeneration, ensuring also antibacterial properties against drug-resistant pathogen infection and, at the same time, promoting bone regeneration, *e.g.* solving the issues of bone deformities.²⁶³ Similar multifunctional BGs were synthesized by Bano *et al.* by co-doping with silver and strontium.²⁵⁵ These Ag- and Sr-co-doped BGs (named Ag-Sr MBGNs) were strongly antibacterial against *Staphylococcus carnosus* and *E. coli* bacteria and highly bioactive thanks to the addition of Sr.²⁵⁵

As vascularization is essential for osteogenesis and tissue formation in general, the combined stimulation of both osteogenic and angiogenic activities by strontium- and cobalt-substituted BGs have been shown to successfully promote *in vitro* bone regeneration when seeded with human umbilical cord perivascular cells (HUCPVCs),⁷⁶ which contain a subpopulation that shows common features of the MSC phenotype such as a high frequency of colony-forming unit-fibroblasts (CFU-F).⁷⁶ Thus, these BGs synthesized by Kargozar *et al.*⁷⁶ are optimal candidates as functional bone substitutes.

Angiogenesis was proved to be enhanced by the synergical effect of different couples of pro-angiogenic ions, such as boron and cobalt, cobalt and copper,²² and copper and zinc.²¹⁵ The synergical effect of B and Cu has been reported in the case of B- and Co-doped 45S5 Bioglass® by Chen *et al.* in 2020, showing that the secretion of VEGF from bone marrow-derived stromal ST-2 cells was increased by the dual-doped BG in comparison with single (B or Co) doped BG.⁶⁶ The synergy of the ionic dissolution products (Cu²⁺ and Co²⁺) from Cu- and Co-co-doped BGs has been shown to enhance wound healing ability, not only *in vitro*, but also *in vivo*. After being implanted, these microfibrillar constructs induce better neovascularization, re-epithelization, and ordered deposition of ECM components (such as collagen and elastin) of damaged skin in the dorsum of rabbits, in comparison with commercial wound dressings.²²

Preliminary studies of the bioactivity and cytotoxicity to Saos-2 cells of Cu- and Zn-co-doped BGs (1Cu1Zn-NaBG) have been also carried out, to evaluate the feasibility of co-doping with these two ions for potential use in bone regeneration thanks to the combination of the antibacterial properties and regenerative therapeutic effects of Cu (angiogenic agent) and Zn (osteogenic agent).²⁶⁴

Borate glasses have been doped with 6 mol% and 9 mol% of Sr, enhancing the cell growth rate, the ALP activity, and the bone sialoprotein (BSP) and osteocalcin (OCN) mRNA level of

MSCs, thanks to the combined effects of boron and strontium ions, that improved the osteogenic ability of these glasses.²⁶⁹ In this context, it should be underlined that concentrations of boron above 0.65 mM may inhibit the growth and proliferation of MG-63 cells. The presence of SrO (0–9 mol%) in the composition (mol%) of borate-based 13-93B2 glass scaffolds (18SiO₂–36B₂O₃–22CaO–6Na₂O–8K₂O–8MgO–2P₂O₅) can accelerate the release of Si⁴⁺ and Ca²⁺ ions and suppress the rapid release of B³⁺, thus significantly improving the cell proliferation and reducing the cytotoxicity of glass scaffolds.⁵⁵

Khan *et al.*²⁶⁵ demonstrated the synergical osteogenic effect of Sr and Li ions. Indeed, significant new bone formation, an abundant collagenous network, and a minimal or no interfacial gap between the bone and the implant were observed in Sr-doped and Li- and Sr-co-doped samples as compared with Li-doped samples.²⁶⁵ In addition, Li- and Sr-co-doped (Li + r) samples led to an enhanced formation of peripheral cancellous tissue on the periphery and cortical tissues inside the implanted samples and to a higher degree of vascularity in comparison with the other tested glass compositions.²⁶⁵

Analogously, co-doping with cobalt and strontium has shown synergic osteogenic effects both *in vitro* and *in vivo*, showing *in vitro* calcium nodule formation and the expression of osteogenic markers and better bone healing when implanted in critical size defects of the distal femur in rabbits in the case of the group receiving Sr- and Co-co-doped glass constructs, compared with the Co-free groups, at both 4 and 12 weeks of follow-up.

In addition, it has been demonstrated that co-doping with two antibacterial ions like Ag and Li ions leads to an enhancement of the antibacterial properties of the modified BG. Four Li- and Ag-co-doped BGs (LA-BGs) with a fixed content of lithium (5 mol%) and a variable amount of silver (*i.e.*, 60SiO₂–31CaO–4P₂O₅–5Li₂O–0Ag₂O, 60SiO₂–30CaO–4P₂O₅–5Li₂O–1Ag₂O, 60SiO₂–26CaO–4P₂O₅–5Li₂O–5Ag₂O, and 60SiO₂–26CaO–4P₂O₅–5Li₂O–10Ag₂O) have been synthesized *via* a sol-gel technique, demonstrating that the BGs doped with 1 mol% of Ag and 5 mol% of Li realized the best cell proliferation and, at the same time, the best antibacterial properties.¹⁹²

A synergistic antimicrobial efficacy of different antibacterial ions has been demonstrated also in the case of Cu- and Co-doped phosphate glasses at a concentration of 5 mg mL^{−1} against *E. coli* in comparison with single-doped glasses.²⁶¹

In 2023 Taye *et al.* published the first (based on a literature overview of the work's authors) Ag- and Ce-co-doped BG (80S–Ag–Ce–MBG),²⁵⁷ proving the antibacterial efficacy of this novel glass formulation against *E. coli*. These results confirmed the ones published by Raja *et al.* in 2022, showing a synergistic antimicrobial effect of Cu and Co against *E. coli* and Cu and Zn against both *E. coli* and *S. aureus*.²⁶¹

The successful treatment of full-thickness injuries in rabbits by eggshell membranes coated with a Zn- and Co-co-doped glass demonstrated the synergistic effect of cobalt with zinc on wound closure efficacy as compared with the effect of single ions.²⁶⁷

Besides the abovementioned applications, the co-doping of BGs has been also studied to better control the sintering

process. A study published in 2019 by Ben-Arfa about the effects of Cu²⁺ and La³⁺ doping on the sintering ability of sol-gel derived high-silica BGs revealed that copper promotes early densification, enhancing the density at lower sintering temperatures, and ultimately crystallization, whereas lanthanum offers beneficial effects at sintering temperatures higher than 1000 °C, inhibiting crystallization.

In the case of co-doping, an interesting novel method for the dual addition of doping ions was developed by Li *et al.*, as reported in 2022.²⁶² They employed the scalable reactive laser fragmentation in liquids method to produce 45S5 BG nanoparticles in the presence of light-absorbing Fe and Cu ions, successfully doping these nanosized BGs with two metal ions up to 4 wt%.

Another interesting approach to fabricating multifunctional BGs is to combine the doping with therapeutic and/or antibacterial ions with the loading with therapeutic and/or antibacterial drugs.

The synergetic effect of antibiotic drugs and antibacterial ions is, indeed, under investigation, as shown by several experimental works.^{214,270}

Porous Li-modified BG nanoparticles were loaded with vancomycin or 5-FU to allow multiple deliveries of lithium ions with osteogenic properties and drugs with antibacterial or antitumoral properties.²²⁵ So, these BGs could be used for curative and restorative bone treatment, in particular, bone regeneration as a consequence of the bioactive properties of the BG nanoparticles themselves, osteoporosis treatment stimulated by the doping with Li ions, osteomyelitis treatment or cancer treatment in the case of vancomycin or 5-FU controlled release.²²⁵

Another promising candidate for bone tumor treatment is Se-doped and doxorubicin-loaded mesoporous sol-gel BG particles, because of their very good bioactivity (characterized by HA formation after only 3 days in SBF) and sustained and controllable drug delivery.¹²⁰ The addition of small amounts of selenium into the mesoporous BGs did not alter their mesoporous structure but enhanced both the drug-loading ability and the drug-release kinetics.¹²⁰

Remaining in the application field of anticancer therapy, in particular for the therapy of osteosarcoma (a common malignant bone tumor, generally treated by surgical resection), Nd-doped mesoporous borosilicate bioactive glass-ceramic bone cement was developed for the repair of the bone defects caused by the surgery and the synergistic therapy of osteosarcoma recurrence through the combination of photothermal therapy and release of doxorubicin.²²⁶ The sol-gel Nb-doped mesoporous bioactive glass-ceramics microspheres act both as a photothermal agent and a drug carrier, resulting in a bone cement with an enhanced killing effect on MG-63 osteosarcoma cells thanks to the synergistic effect of photothermal and chemical therapies.²²⁶ The photothermal properties were given by the presence of Nd³⁺ whereas the drug-loading ability was by the mesoporous structure of the glass-ceramics.²²⁶ It is interesting to point out that the increase in temperature (caused by the photothermal therapy) significantly accelerated



the release of doxorubicin from this drug-loaded bone cement.²²⁶

In 2020, copper doping has been demonstrated to influence the lattice of BGs, and consequently, their morphology and porosity, which influence the ionic dissolution (hence modulating glass bioactivity) and drug release in the case of porous Cu-doped BGs.²⁷¹ Due to the mesoporous network, 1.5% and 2.5% Cu-doped BGs showed an enhanced release of anti-inflammatory drugs such as acetaminophen and ibuprofen.²⁷¹ Thus, these Cu-doped BGs act as antibacterial drug carriers and are characterized by antibacterial properties amplified by the presence of copper, being potentially able to up-regulate the healing properties in dental applications and control oral microbial exposure.²⁷¹

Also, cerium influences the porosity of mesoporous BGs; in particular, Farag *et al.* showed that the pore size diameter of MBGs decreased as the cerium content increased in all MBGs.²⁷² Doping with Ce ions was also proved to enhance drug adsorption (in detail, vancomycin).²⁷² Ce-free MBGs showed, in fact, the lowest drug absorption and the highest drug release percentage.²⁷² Thus, the actual MBGs were considered promising candidates for bone regeneration and bone cancer treatment, possessing the added value of being able to reduce the bacterial activity around the tumor site.²⁷²

The dependence of drug loading efficiency on the content of the doping ion was confirmed also by experimental studies of Mg-doped BGs,⁵⁵ which were modified to introduce amino groups on their surface, with the aim of loading negatively charged biomolecules such as amoxicillin.⁵⁵ It was proved that amoxicillin loading slightly decreased with an increase of Mg²⁺ content (from 0 mol% to 5 mol%) in the BG composition.⁵⁵

The above-reported studies^{271,272} demonstrated that drug loading and drug release could be controlled by the glass composition.

Drug release can be tracked and monitored using luminescent BGs on the basis of the change of the luminescence intensity, such as Eu³⁺/Tb³⁺-doped mesoporous electrospun BG nanofibers (MBGNFs) with an average diameter of 100–120 nm.²⁷³ If loaded with ibuprofen, these luminescent nanofibers were shown to be able to release it in a sustained way *in vitro*, and biocompatibility tests on L929 fibroblast cells using the MTT assay have revealed the low cytotoxicity of MBGNFs.²⁷³

Finally, an interesting work on doping with different ions and its synergical use with the release of vitamin E was published by Hu *et al.*²⁷⁴ Borate BGs were doped with copper to mitigate issues related to the uncontrolled release of borate glasses, which can lead to a very rapid degradation and transient biotoxicity;²⁷⁴ in fact, the addition of Cu in borate glasses has been proved to render borate and borosilicate glasses more stable and to lower the release of other ions, such as Ca and B (although Cu-doped 13-93-BS glass showed an enhanced release of Si, probably because of the preferential connection of the modifier B ion in the network structure of borosilicate glasses to Si, making the Si–O–Si bonds more breakable).^{258,275} They were used for the fabrication of a novel organic–inorganic

dressing of BG/poly(lactic-co-glycolic acid), which was loaded with vitamin E (named VE-Cu BG/PLGA).²⁷⁴ The *in vitro* cytotoxicity and angiogenesis of the VE-Cu BG/PLGA dressing were assessed using human fibroblasts and umbilical vein endothelial cells (HUVECs), showing positive pro-angiogenic effects in both cases: fibroblasts expressed higher levels of angiogenesis-related genes and HUVECs showed high migration, tubule formation, and VEGF secretion.²⁷⁴ *In vivo*, in the case of full-thickness skin wounds in rodents, a significant improvement in the epithelialization of wound closure, and a clear enhancement in vessel sprouting and collagen remodeling was observed.²⁷⁴ Thus, this dressing was considered very promising for the stimulation of angiogenesis and reconstruction of full-thickness skin defects, thanks to its controlled release of both Cu²⁺ and vitamin E.²⁷⁴

Functionalization and coatings of bioactive glasses

BG surfaces can be further chemically modified with various biomolecules (such as polymers, proteins, vitamins, and drugs), imparting or improving bioactive, mechanical, regenerative, and/or antibacterial properties.

Various strategies of chemical surface modification are available and the most suitable and effective in the case of BGs involve the use of coupling agents or pH changes; other approaches involving the deposition of coatings, the use of radiation, and the development of core–shell systems are also possible and widely investigated.²⁸⁴ Moreover, the ion exchange method can be used to modify the bioceramic surface, as reported by J. H. Lopes *et al.* who adopted a molten salt bath (Ca²⁺ molten salt bath|Na⁺ glass) immersion ion exchange process to selectively change the surface reactivity and enhance the bioactivity of a melted 45S5 Bioglass® by accelerating some reactions in the early stages of Hench's mechanism.²⁸⁵

To improve the mechanical stability of highly porous glass and glass-ceramic scaffolds, many authors have coated the scaffolds with biodegradable polymers.^{286–288} An ideal scaffold for bone TE should, in fact, offer suitable mechanical properties, adequate pore size, a high degree of porosity (to enable tissue ingrowth, vascularization, osteoconductivity, and osteoinductivity), and an adequate degradation rate, but most bioceramic scaffolds are too brittle and not suitable for load-bearing applications.²⁸⁷

Moreover, BG and bioactive glass-ceramics can be functionalized in order to enhance their biocompatible and bioactive properties²⁸⁶ (referring in this case to the general meaning of the term bioactivity as the ability to induce or modulate biological activity⁵⁶ and not only their ability to bond to bone). Such coatings are able to provide a high degree of biocompatibility and promote cell adhesion²⁸⁸ and tissue healing without causing severe reactions.²⁸⁷

Biologically active molecules and ECM proteins/peptides can be grafted on the BG surface to stimulate physiological



bone regeneration with both inorganic and organic signals.²⁸⁶ For example, glass-ceramic scaffolds with very high mechanical properties and moderate bioactivity have been successfully functionalized with ALP, which is involved in bone formation and mineralization and is widely used as a marker of osteoblast differentiation in *in vitro* tests.²⁸⁶

Furthermore, the ECM possesses transmembrane integrin receptors with extracellular domains that can recognize motifs such as the sequence Arg-Gly-Asp (RGD) within ECM adhesion proteins, *e.g.* fibronectin and vitronectin, and cytoplasmic domains that can interact with proteins like vinculin, paxillin, talin and actin, but also with other signaling molecules (such as FAK and c-Src) at the focal adhesion sites.^{28,289} So it is possible to graft ECM adhesion proteins or derived peptides²⁸ and three main grafting techniques are available: simple adsorption, covalent bonding, or release from a degradable carrier.²⁹⁰

Growth factors such as bone morphogenetic proteins, insulin-like growth factors (IGF), TGF- β , and FGF can also be linked to the BG surface, imparting enhanced osteogenic properties to the bioactive glass.²⁹¹

BGs and glass-ceramics are particularly reactive and prone to surface modifications, not only by direct anchoring, but also through functionalization with aminosilanes thanks to numerous silanol groups available on their surface.²⁸⁶ Thus, the BG surface can be functionalized using several functional groups, that can act as active sites for specific properties or further molecular grafting, as recently well reviewed by Kargozar *et al.*²⁸⁴ As shown by many experimental studies,^{284,290,292–299} the functional group most commonly introduced on the surface of BGs is the amino group (NH₂); 3-aminopropyltriethoxysilane (APTES, C₉H₂₃NO₃Si), a nontoxic protein-coupling agent that can be easily introduced using silanization, was proposed by Verné *et al.*³⁰⁰ as a successful agent for covalently bonding bone morphogenetic proteins (*e.g.*, BMP-2) to the BG surface, as confirmed by several experimental results (as reported below). For example, to improve their ability to bond with biomolecules, such as drugs, proteins, and peptides, gallium-containing BGs, characterized by the composition 45.7SiO₂–24.1Na₂O–26.6CaO–2.6P₂O₅–1.0Ga₂O₃ (Ga1.0), were successfully functionalized with tetraethyl orthosilicate (TEOS) and APTES.³⁰¹ Novel highly porous 45S5 Bioglass®-based scaffolds have been fabricated by Rezwan *et al.*²⁹² using the polymer replica technique and later surface-functionalized with APTES and glutaraldehyde without the use of organic solvents, but it is worth noticing that these surface-modified BG scaffolds have shown an enhanced bioactivity only thanks to their more pronounced surface micro-roughness, which was believed to be caused by ion leaching during the functionalization aqueous heat treatment (at 80 °C). The influence of surface roughness on the behavior of bone cells and glass bioactivity will be further discussed in the section “Influence of topography on cell behaviour – superficial and internal nanostructure”.

Despite Rezwan's results, Ferraris S. *et al.*²⁸⁶ have successfully produced ALP-functionalized and highly bioactive

scaffolds of SCNA (57% SiO₂–34% CaO–6% Na₂O–3% Al₂O₃ in mol%) bioactive glass-ceramic by introducing APTES on the glass surface after hydroxyl exposure and silanization of the glass surface itself and the higher bioactivity of these functionalized scaffolds was hypothesized to be related to the ability of the grafted homodimeric metalloenzyme ALP to attract phosphate ions utilizing its Zn cations and thus accelerate calcium ion precipitation and HA nucleation in SBF.²⁸⁶

Zhang *et al.*³⁰² have prepared amino-functionalized MBGs with the composition of 80% SiO₂–15% CaO–5% P₂O₅ (mol%), using the evaporation-induced self-assembly (EISA) method to synthesize the glasses and the reflux agitation method to successfully graft the 3-APTES by the silicon-alkylation reaction. The results indicate that the surface of N-MBG was positively charged, so that surface adhesion proteins with a negative charge could be easily adsorbed on the surface of the N-MBG, promoting the adhesion of mouse embryo osteoblast precursor cells (MC3T3-E1). Moreover, the functionalized MBGs showed an increased surface area and a more rapid release of Ca and Si ions. Therefore, these amino-functionalized ordered MBGs are more conducive to cell adhesion, proliferation, and differentiation compared with BGs and MBGs and so they could be potentially used as platforms for bone regeneration applications.

Furthermore, it is worth noticing that APTES functionalization has been shown to enhance the absorption of metallic ions on the glass surface.²⁹⁷ Analogously, the surface modification of M58S glass with KH550 can improve the Ag⁺ loading capacity (achieved using the dipping method).¹⁹³

Moreover, only when gold is present in the glass composition is it possible to alternatively and selectively form two types of bond with either the amino or thiol groups of small organic biomolecules (*i.e.*, either Au–S or Au–N), by simply raising or lowering the temperature of the batch of functionalization, as demonstrated by Aina *et al.*, who have selectively functionalized mesoporous sol-gel silicate BGs containing AuNPs (SiO₂–CaO–P₂O₅–Au₂O), with 2-mercaptoethanol, 2-ethanolamine, and the SH-containing amino acid cysteine, carrying either/both amino or/and thiol groups.³⁰³

It is also possible to impart or improve the antibacterial properties of the implant; the BG scaffold can be coated with antibiotic drugs or antibacterial metallic ions, such as silver.^{304–307}

Polyphenols are gaining a lot of attention thanks to their antioxidant, anti-inflammatory, antibacterial, bone-stimulating, and anticancer properties.^{308–312} Among other studies, a few years ago, two silicate BGs – SCNA (57% SiO₂–34% CaO–6% Na₂O–3% Al₂O₃) and CEL2 (45% SiO₂–3% P₂O₅–26% CaO–7% MgO–15% Na₂O–4% K₂O, mol%) – have been successfully coupled with gallic acid and natural polyphenols extracted from red grape skins and green tea leaves by Zhang *et al.*^{309,313} and Cazzola *et al.*,³⁰⁸ after surface treatment for superficial –OH exposure. These studies showed that the amount of gallic acid was significantly higher on CEL2 than on SCNA (according to a preliminary test of the Folin & Ciocalteu assay) and that polyphenol-functionalized CEL2 was characterized by a faster HA kinetics deposition (if compared with non-functiona-



Ceramic	Ligand	Functionalization	Bioactivity	Cell response	Antibacterial properties	Ref.
45S5 Bioglass®	APTES and enzyme ALP	Both <i>via</i> silanization and through direct anchoring to hydroxylated surfaces	Enhanced, in particular in the case of direct anchoring of ALP			296
SCNA and CEL2 glasses	APTES and enzyme ALP	Both <i>via</i> silanization and through direct anchoring to hydroxylated surfaces	Enhanced, in particular in the case of direct anchoring of ALP	Stronger expression of one of osteocalcin, good cell viability, and proliferation		290 and 315
MBGs (85% SiO ₂ -15% CaO, mol%)	APTES	Amination and then chemical links with amino acid sequences of collagen, to synthesize collagen hydrogels incorporating NH ₂ -functionalized MBG NPs	Improved	Excellent viability, good proliferation, and osteogenic differentiation, enhanced cytoskeletal extensions of MSCs cultivated on Col-mBGn hydrogels		293
BG with composition (mol%) 80SiO ₂ -15CaO-5P ₂ O ₅	APTES	Amino functionalization by reflux agitation method	Good	Good platform for MC3T3-E1 cell adhesion		302
BGs belonging to the 56SiO ₂ ·(40 - x)CaO·4P ₂ O ₅ ·xAg ₂ O system (with x = 0, 2, and 8 mol%)	APTES and protein coupling agent glutaraldehyde	Before protein attachment, silanization with APTES, and surface functionalization with glutaraldehyde		Improved stability of the protein attachment, reduction amount of the adsorbed protein (balanced by silver presence), and good hemoglobin affinity		295
Ga1.0	TEOS and APTES	Silanization with APTES and then functionalization with TEOS	After a short SBF soaking time (4 days), separation of calcite favored by APTES functionalization whereas separation of HA favored by TEOS functionalization			301
Highly ordered mesoporous 58S Bioglass® (SM58S) and Ag-doped MBGs (Ag-SM58S)	γ-aminopropyl triethoxy-silane (KH550)	Direct grafting		<i>In vitro</i> osteoblast proliferation and differentiation	Against <i>E. coli</i> and <i>S. aureus</i> (thanks to the sustained release of Ag ⁺)	193
45S5 Bioglass®	Silver ions	Immersion into a silver nitrate solution, followed by the addition of formaldehyde and then 8% ammonia solution	Acceleration of the nucleation and growth of highly crystalline HA driven by the presence of AgNPs on the surface		Not tested, but supposed because of the presence of Ag ⁺	305
BG with composition 0.42SiO ₂ -0.15CaO-0.23Na ₂ O-0.20ZnO	Silver ions	Immersion into a silver nitrate solution			Against <i>Staphylococcus epidermidis</i> and <i>E. coli</i>	306
SCNA (57% SiO ₂ , 34% CaO, 6% Na ₂ O, and 3% Al ₂ O ₃)	Silver ions	Thermo-chemical treatment to induce ion exchange			Against <i>S. aureus</i> and <i>E. coli</i> with a zone of inhibition of about 2 mm	304
BG with composition 50SiO ₂ -18CaO-9CaF ₂ -7Na ₂ O-7K ₂ O-6P ₂ O ₅ -3MgO	Silver ions	Ion exchange	Good	Good biocompatibility	Good	307

Table 2 (Contd.)

Ceramic	Ligand	Functionalization	Bioactivity	Cell response	Antibacterial properties	Ref.
CEL2	Polyphenols from <i>P. pavonica</i> algae and AgNPs surface	<i>In situ</i> reduction			Against <i>S. aureus</i> , however, a certain specimen toxicity was observed toward human progenitor cells	312
Bioactive glass (similar to the 45S5 Bioglass®) in the system SiO ₂ –Na ₂ O–CaO–P ₂ O ₅ was prepared, using a standard glass-forming process by mixing SiO ₂ , Na ₂ CO ₃ , CaCO ₃ , and CaHPO ₄ in the following weight ratio 33.67 : 31.34 : 26.38 : 8.61	APTES and rhBMP-2	Exposure of –OH groups, functionalization with APTES, surface activation with carbonyldiimidazole, and finally immobilization of rhBMP-2 recombinant		Non-covalently immobilized rhBMP-2 is biologically highly active, whereas covalently bound protein is not, although juxtracrine stimulation is supposable		298
45S5 Bioglass®	APTES and collagen	Functionalization with APTES and then coating with collagen	Higher cell viability (MG-63 human osteosarcoma cell line) on cross-linked collagen-coated samples	Collagen offers many nucleation sites for HA formation		299
Silicate (S53P4) and phosphate (Sr50) BGs	APTES	Basic treatment and silanization for fibronectin absorption	Improved cell adhesion and promotion of cell spreading			289

Therefore, the extracellular matrix of cells provides structural support and bioactive cues to cells for the regulation of their activities. Adherent cells possess, indeed, thin ECM projections (called filopodia, lamellipodia, and nanopodia), that permit the cell itself to move and sense the surface topography during adhesion. These projections end with cell attachment molecules that serve as anchor points for movement. The most common are specific receptors called integrins, heterodimeric proteins constituted of two subunits, α and β , which cluster together and recruit cytoplasmic proteins to form a focal contact and adhere to the surface, developing a defined cytoskeleton.^{332,333} Surface structure, in particular its nanoscale features (dimensionally comparable with the focal adhesions of the cells, whose size is in the range of 5–200 nm), influences cell adhesion affecting the recruitment of integrins (characterized by a size of 8–12 nm) and consequently cytoskeleton development. This in turn affects cell differentiation, proliferation, spreading, signal transduction pathways, and survival.^{10,30} Integrins sense the biophysical and biochemical properties of the ECM and are linked to the nucleus directly through the cytoskeleton and indirectly through signal transduction, actuating the abovementioned mechano-transduction pathways. Thus, these receptors are involved in mechanosensing and bi-directional transmission of mechanical force, but if the spacing between the integrins is lower than 70 nm, the integrins cannot gather together and focal adhesion does not take place.

The organization of the ECM is frequently hierarchical from nano- to macro-features, with many proteins forming large-scale structures with feature sizes up to several hundred microns.²⁹ The nanoscale structure of the ECM, constituted by a natural intricate nanofibrous web, supports cells and presents an instructive background to guide their behavior, paving the way for cells to form complex tissues such as bone, liver, heart, and kidneys.²⁸ Engineered nanoscale features are orders of magnitude smaller than most mammalian cells, *e.g.* osteoblasts, and are comparable with the integrins, as discussed above, and can mimic the irregularities in the ECM (such as its undulations, bending, branching, *etc.*), and on the cell membrane.³³⁴ Therefore, nanoscale patterning can influence the organization and type of the forming focal adhesions, either by disrupting their formation or by inducing specific integrin recruitment, influencing the morphology of the cells and allowing the formation of regions with or without cell colonization on a biomaterial surface,²⁸ whereas micro-features can regulate the cell behavior at the level of cell populations or at the level of a single cell.²⁸ For example, the implant's superficial roughness can affect the production of local growth factors and cytokines by osteoblast-like MG-63 cells and, consequently, modulate their cell activity.¹¹

On the other hand, the macro features of the surface (in the range of hundreds of μm) do not influence the cell adhesion and spreading, because the cells usually spread only over distances of tens of μm , and thus they can spread both on the



Osteoblasts are the largest of the mesenchyme-derived cells and require a high degree of intracellular tension to support the expression of a phenotype. The osteocytes of bone tissue are considered the main mechanosensing cells of bone, the cellular units responsible for the transduction of the mechanical or physiological stimuli into a differential cellular and tissue response.^{9,104} Modification of the surface topography through micro-roughening has been shown to enhance the osseointegration of biomedical implants in orthopedics and dentistry, such as titanium implants and BG scaffolds.^{10,11,337} Indeed, as shown by Pfössl *et al.*³³⁸ osteoblast-like MG-63 cells oriented in the direction of the grooves, which acted as contact guidance. Moreover, Karazisis *et al.*³³⁹ have shown that the controlled nanotopography of titanium implants downregulated the expression of monocyte chemoattractant protein-1 (MCP-1), from day 1 of cell culture, and triggered the expression of osteocalcin at day 3, promoting higher osteogenic activity and a larger amount of early formed osteoid and woven bone. In the case of etched micro- or nanostructured BG surfaces, it was stated by Koort *et al.*³⁴⁰ that the leaching of Na, K, Mg, P, and Ca ions and the enrichment of the glass surface with Si ions could have a significative positive effect on the bone regeneration caused by etched BGs due to the enhanced glass solubility. Moreover, they observed the growth of cortical bone in the interstices of the BG implant, which could lead to an accelerated osteointegration of the bone substitute.³⁴⁰ Regarding the osteointegration, Wang *et al.*³⁴¹ have obtained good results *in vitro* in terms of the adhesion, spreading, and proliferation of HOB cells by covering the surface of orthopedic titanium implants with a nanostructured glass-ceramic coating (as described in Table 3). Moreover, the release of Ca and Si ions from the glass coating stimulated the expression of genes related to the bone tissue.³⁴¹ A strong bonding between the bone and implant can be further

However, despite the intense experimental and theoretical investigation into understanding the physical, chemical, and biological aspects of cell-surface interactions, the knowledge of these phenomena is mostly limited to the interactions on the macro- and micro-length scales, with only limited information about cell behavior in contact with surface nanoscale features, and the mechanisms by which surface topography

Glass composition	Nanostructuring method	Final material	Application	Ref.
60% SiO ₂ , 36% CaO, 4% P ₂ O ₅ (mol%)	Acid-catalyzed sol-gel synthesis using citric acid (CA) as the catalyst and TEOS as the silica precursor, to control surface nano-morphology	Bioactive nanopatterned glass, with high <i>in vitro</i> bioactivity and the ability to promote adhesion and proliferation of MSCs (in particular three morphologies were obtained: (1) surface covered by particles in the size range 100–300 nm if CA/TEOS = 0.11, (2) nanoporous surface covered by nanoparticles if CA/TEOS = 0.22, (3) surface covered by dispersed particles if CA/TEOS = 0.33)	Bone TE	356
Borosilicate glass with low alkali content	Combination of a physical vapor deposition method (radio-frequency magnetron sputtering) on a melted silica substrate with a metastable phase separation synthesis, followed by differential etching of the coating	Glass phase-separated nanostructured film with a spinodal structure	Optical field	357
Biocompatible glass belonging to the system CaO–Na ₂ O–P ₂ O ₅ –SiO ₂	Melting, crystallization of different phases (spinodal decomposition and phase separation), and finally selective chemical leaching (depending on the variable SiO ₂ content and the performed thermal treatment for glass crystallization)	Spinodal microstructured glass with multimodal porosity	Bone TE	358
Bioactive glass with the following composition: 24.4% Na ₂ O–26.9% CaO–2.6% P ₂ O ₅ –46.1% SiO ₂ (mol%)	Melting; crystallization at the microscale level (spinodal decomposition and multiple phase separation), selective chemical leaching	Spinodal microstructured glass with multimodal porosity	TE scaffolds	359
Quartz	Wet chemical etching of a coating composed of a monolayer of perfluorodecyl trichlorosilane (previously deposited on a glass substrate using LPCVD at 620 °C, to create nanostructures on the substrate) and thermal oxidation of the formed silica nanostructures (no need for mask removal)	Nanostructured super-hydrophobic and transparent glass with anti-fog properties	Optical field	360
Optical glass (L-BAL42, OHARA)	Dry etching (reactive ion etching, RIE) with a nano-mask formed by ultrathin film (<10 nm of thickness) sputtered on the glass substrate, leading to the formation of pillars	Hierarchical micro- and nano-structured glass with anti-reflective and antifog properties	Optical field	361
Soda-lime glass	Non-lithographic, anisotropic etching: (1) deposition of a layer of SiO ₂ on a sacrificial layer of glass by plasma-enhanced chemical vapor deposition (PECVD) using a mixture of N ₂ O and SiH ₄ , (2) CF ₄ plasma treatment (so a super-hydrophilic nanostructure with a high aspect ratio nanopillar of SiO ₂ that acts as a mask for etching), (3) selective etching (slow where there are pillars, faster in zones without pillars)	Nanostructured super-hydrophobic glass (after plasma etching higher roughness, from 0.095 nm to 1.849 after 60 min of CF ₄ plasma etching)	Multifunctional glass surfaces with hydrophobic or super-hydrophilic, anti-fog and low-reflective properties	362
Antiglare glass	Laser-assisted nanoimprinting: (1) quartz layer, deposited using a bipolar-type plasma-based ion implantation and deposition (PBII&D) technique (2) layer able to absorb light (diamond-like carbon), used as a mask for etching followed by reactive ion etching (RIE): 1. deposition of a film of Al on the DLC mask 2. spin-coating of a resist on the film of Al 3. patterning using electron beam (BE) lithography 4. etching of the film of Al by RIE using CHF ₃ gas 5. etching of the layer of DLC using a mixture of O ₂ and Ar (the remaining Al acts as a mask) 6. HCl bath to remove residuals	Nanopatterned glass formed by concave and convex zones	Optical field	363
Silicon nitride, zirconia and 45S5 Bioglass®	High-energy laser treatment of surfaces of silicon nitride (β-Si ₃ N ₄) and zirconia-toughened alumina (ZTA) to create a series of regular cylindrical cavities, which were subsequently filled with 45S5 Bioglass® powders, mixed with different fractions of Si ₃ N ₄ powders (0, 5, and 10 mol%) – the addition of 45S5 Bioglass® was fundamental to render this material bioactive	Bioactive functionalized double ceramic microstructured surfaces formed by a series of regular, cylindrical cavities filled with 45S5 Bioglass® powders and Si ₃ N ₄ powders due to the combined effect of laser patterning and Bioglass®/Si ₃ N ₄ filling	Bone implants	364
Borofloat glass	Non-lithographic process: (1) deposition of a nickel film by electron beam evaporation (annealing at 600–650 °C per 5 min to form nickel nanoparticles on glass surface), (2) etching using these nickel NPs as a mask and SF ₆ plasma in an inductively coupled plasma (ICP) etching system	Nanostructured glass with self-cleaning properties	Solar cell packaging	365
GDC/YSZ system	GDC sputtering using Discovery 18DC/RF magnetron sputter deposition system: (1) thin films of sputtered GDC on YSZ substrates, (2) annealing	Nanostructured hydrophilic surface, characterized by various nanopatterns depending on the annealing treatment: isolated isles, connected isles, and pits, with width variable in the range 75–250 nm and height variable in the range 100–2500 nm	Bone TE (<i>e.g.</i> orthopedic implants) and soft TE (<i>e.g.</i> corneal regeneration if printing on hydrogels)	318

Glass composition	Nanostructuring method	Final material	Application	Ref.
45S5 and 13-93	Direct mold casting	Periodical microtexture (groove of 10 μm)	Bone TE	338
Glass with low T_g	Imprint lithography using a mold of $\text{Si}_3\text{N}_4/\text{SiO}_2/\text{Si}$ and a glass with low T_g Steps: 1. molding in a silicon-based mold stamp produced by the VLSI process (a) deposition of a layer of SiO_2 on a substrate of Si (low-pressure chemical vapor deposition) (b) etching by reactive ion etching 2. mold pressed on the glass substrate	Glass with surface parallel gratings (some of them even in the micrometric range), forming a pattern of 1.0 mm of length 330 nm or 250 nm of width, and 300 nm of depth	Optical field (optoelectronics)	366
Powders of zirconia reinforced with alumina (ATZ) – composed of 3% of ZrO_2 stabilized with yttrium with circa 20% of Al_2O_3	Femtolasers for micro-structuring the surface of disks of powders of zirconia Steps: 1. preparation of zirconia powder disks, pressing powders of zirconia with 49 N, pre-sintering them at 1000 $^\circ\text{C}$, polishing and sintering them at 1500 $^\circ\text{C}$ for 2 h 2. femtolasers	Microstructured zirconia	Load-bearing long-term applications in the maxillo-facial and dentistry field	329
45S5 Bioglass®	Melting-quenching of 45S5 Bioglass® and then laser treatment of the glass surface	Nanostructured 45S5 Bioglass® with an increased surface roughness, a bigger surface area, and a more hydrophilic behavior (the water contact angle of BG changed from 80° to 34° for untreated and laser-treated samples, respectively)	Bone TE	342
Powders of glass-ceramics HT ($\text{Ca}_2\text{ZnSi}_2\text{O}_7$) e SP (CaTiSiO_5)	Atmospheric plasma spray	Coating of HT and SP on disks of Ti-6Al-4V	Bone TE	341
3D bioactive glass-ceramic	Modification of the scaffold surface with (A) either buffered water (pH = 8) with APTES at 80 $^\circ\text{C}$ for 4 h (B) or just simply with the buffered water on its own under the same conditions	BG scaffold with nanostructured surface topography, in detail scaffold surfaces with: (A) cavities of almost 599 nm, which were formed by the remaining silicate glass matrix after leaching of the $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ crystals from the scaffold (B) uniformly distributed spindle-shaped submicron-scale crystallites (this indicated a continuous dissolution of both glass and crystalline phases)	Scaffold per TE	367
Three glasses: 19–93 1–98 3–98	Chemical etching with a solution of NH_4F (22 M) and $\text{C}_8\text{H}_{10}\text{O}_7$ (8.5 M) at 1 : 3 (vol%) ratio	Bioactive glass with surface microroughness	Bone and cartilage TE	340
45S5 Bioglass®	Liquid precursor plasma spraying (LPPS) Synthesis of the precursor: 1. mixing of TEOS and EtOH under magnetic stirring (molar ratio 1 : 2) 2. H_2O addition ($\text{TEOS} : \text{H}_2\text{O} = 1 : 8$) 3. addition of an ammonia solution, used as the catalyst for TEOS hydrolysis 4. addition of TEP after 40 min (followed by stirring for 20 min) 5. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and NaNO_3 dissolved in water and then added to the initial suspension 6. stirring for 1 h Plasma spraying using an Metco MN air plasma spraying system and AR2000 thermal spraying robot	Titanium disks coated with a nanostructured BG film	Bone TE	353
Bioactive glasses with compositions (wt%): 13–93, 1–98 and 3–98	Chemical etching with different solutions with pH varying in the range 1–13	Sintered microspheres characterized by surface roughness composed of microgrooves and small pits	Orthopedic implants	368
BG solid disks and BG porous scaffolds	Novel chemical etching method with a solution consisting of NH_4F (22 M) and $\text{C}_8\text{H}_{10}\text{O}_7$ (8.5 M) 1 : 3 (vol%)	Controlled micro-rough surface on BG solid disks and porous implants made of sintered microspheres	Bone TE	11
45S5 Bioglass®	Variation of melting temperatures and thermal treatments	45S5 Bioglass® with spinodal or droplet-like morphologies	Bone TE	336



Anyway, to maintain a critical point of view, it is necessary to mention the intrinsic limitation of these nanostructured

Another very interesting patterned deposition of bioactive glasses is the micropatterning of bioactive glasses on free-

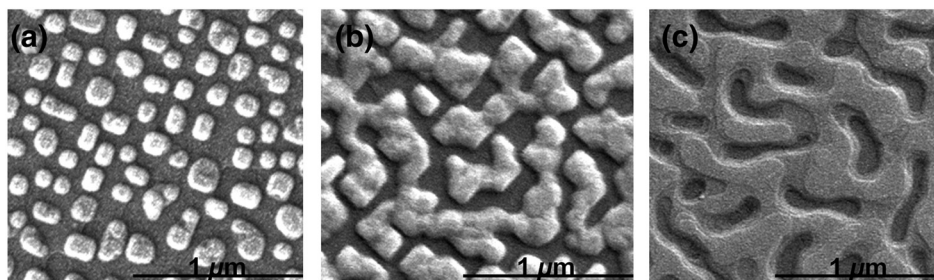


Fig. 3 Scanning electron microscopy of the patterns (nanofeatures) obtained from selectively chemically etching bioactive glass solid disks and porous scaffolds manufactured using the GDC/YSZ system: (a) islands, (b) connected islands, and (c) pits. Scale bar of each micrograph equal to 1 μm . Reprinted from ref. 318 (Copyright © 2012) with permission from Elsevier.

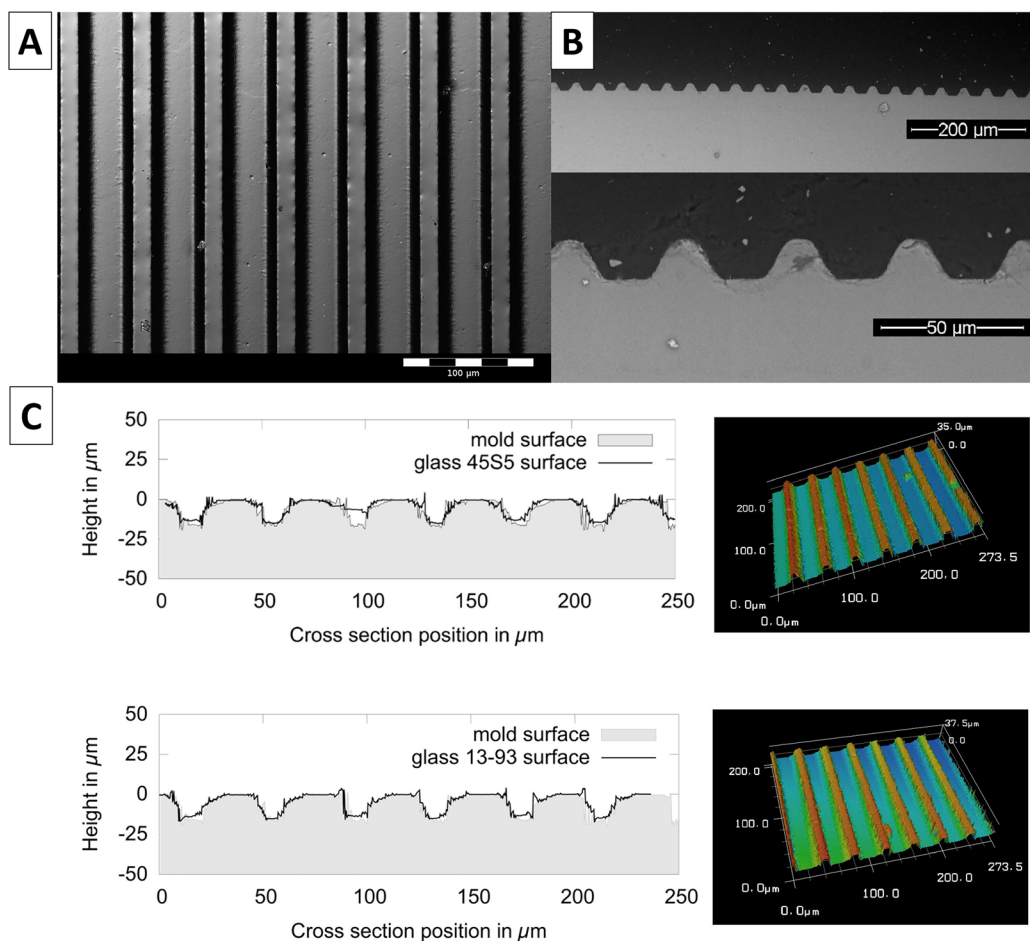


Fig. 4 Microstructured 45S5 Bioglass® and 13-93³³⁸ with permission of DeGruyter: (A) surface of glass 13-93; (B) SEM micrographs of the cross-section of cast bioactive glass 45S5 showing the surface microstructure; (C) 2D laser scanning profile of the surface microstructure on bioactive glass 45S5 (top) and 13-93 (bottom) superimposed with a 2D scan of the mold surface, as well as 3D laser-scanning profiles of the glass surfaces of 45S5 and 13-93 (right).

standing chitosan membranes, achieved by Luz *et al.*,³⁵⁵ who patterned BG sol-gel nanoparticles with a composition (mol%) 55SiO₂-40CaO-5P₂O₅ on free-standing chitosan membranes by microcontact printing using a poly(dimethylsiloxane) (PDMS) stamp. Firstly, they inked the PDMS stamp in a glass substrate covered with a homogeneous layer of BG nano-

particles; then they pressed the PDMS stamp covered with BG nanoparticles on the surface of the chitosan membranes, which were used as a printing substrate (Fig. 5A³⁵⁵). Thus, they were able to spatially control the properties of biomaterials at the microlevel, inducing a patterned growth of hydroxyapatite on the chitosan substrates (Fig. 5B³⁵⁵), and their novel



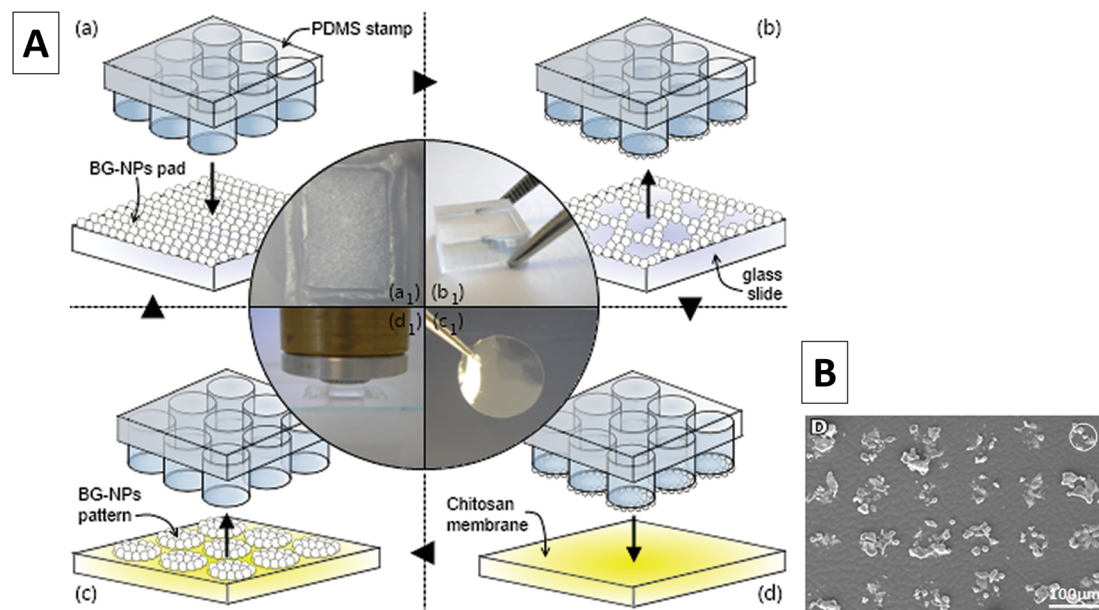


Fig. 5 (A) Schematic illustration and photographs of the fabrication process of the micropatterned bioactive glass deposition on self-standing chitosan membranes: (a) inking of the PDMS stamp in a glass substrate covered with a homogeneous layer of BG-NPs (a1); (b) lift-off of the PDMS stamp carrying the BG-NPs on the base of the features and PDMS stamp (b1); (c) pressing of the PDMS stamp into the chitosan membrane's surface, and the chitosan membrane used as printing substrate (c1); (d) printing of the BG-NPs over the chitosan membrane's surface, and detail of the device used to press the stamp against the substrate (d1). (B) BG-NPs patterned membranes and micropatterned HA formation. Reprinted (adapted) from ref. 355 (Copyright © 2012 American Chemical Society).

composite membranes are believed to be potentially able to guide tissue regeneration for skin, vascular, articular, and bone tissue engineering and in cellular cocultures, or to confine cells in regions with controlled geometry at the cell's length scale.

Up to now, we have described the influence of the external surface micro- and nanotopography, but it is also possible to identify bulk micro- and nanotopography.

Indeed, an internal nanostructure can be observed in the case of porous scaffolds. In a scaffold, three different length-scale structures can be identified: the macrostructure, the microstructure, and the nanostructure.³³² In the case of porous scaffolds, characterized by a porous structure, these three length-scale structures correspond to different porosity levels. At the minimum level, pores below 100 μm impart to the scaffold a specific surface roughness which enhances the cell attachment. The second level (pores of 100–500 μm) is related to bone in-growth and vascularization, but also to a decrease in the mechanical properties, in particular in the Young's modulus (that reduces the stiffness mismatch with the native tissue and the associated risk of stress shielding, in the case of BG scaffolds). The third level of pores, above 500 μm in size, are called giant macropores and can be used for the passage of suture wires to anchor the implant to the host bone.⁴⁴ The ideal scaffold should possess a 3D architecture with a morphology that is almost identical to that of natural tissue, with above 50 vol% porosity composed of large interconnecting macro-pores of about 150–200 μm .²⁸⁶ Three-dimensional structures are preferred in comparison with bi-

dimensional ones, because an instructive three-dimensional environment is fundamental for tissue regeneration, as highlighted by Stevens *et al.* in their review.²⁸ Recently, it has been discovered that cells often show a non-natural behavior when they have moved away from their natural niches and seeded onto flat substrates.³²⁸

Scaffolds can be fabricated using many different techniques, not here reported because out of the intended aim of this review, but the interested reader can read the following cited reviews and books.^{367,369} In particular, in recent years, porous scaffolds have gained much attention and have been produced by lots of research groups, using various materials and applying different fabrication techniques,^{41,190,270,370} allowing even the production of hierarchical structures. Furthermore, several hierarchical glass scaffolds have been successfully produced. Hierarchical materials based on bioactive glasses have been reviewed a few years ago by Baino *et al.*⁴⁴

Finally, to be as inclusive and exhaustive as possible, it is important to draw attention to the interaction of nanoparticulate materials with cells. Indeed, not only nanopatterned and nanofibrous surfaces, but also nanoparticles and nanocomposites are considered nanostructured engineered surfaces, as already elucidated in 2008 in a review by Wei *et al.* about nanostructured biomaterials for tissue regeneration.³⁷¹ An interesting review of nanoscale HA particles for bone TE applications was published in 2011 by Zhou and Lee,³⁷² and therefore here we will focus only on nanoscale BG particles and their composites. BG nanoparticles can be synthesized



using various sol-gel synthesis routes, involving acid and/or basic catalysts, such as traditional acid synthesis,^{161,356,373} basic synthesis (e.g. Stöber synthesis),³⁷⁴ and acid/base co-catalyzed methods,^{108,375} enabling the fabrication of particles with different shapes (irregular, rod-like, spherical and so on) and size. However, despite the numerous possible synthesis strategies, aggregation of nanoparticles often occurs, resulting in a nanostructured architecture composed of lots of aggregated nanostructured particles (briefly called nanoparticles), as shown by some experimental works.^{108,172,375} Thus, because of their size in the nano-range and the nanostructured surfaces created by their aggregation (in other words because of their surface nanostructuring), bioceramic NPs allow improved cellular adhesion, enhanced osteoblast proliferation and differentiation, and increased biomineralization of bone and are attracting increasing interest.²⁹¹ Increased osteoblast (bone-forming cells) adhesion on nanograined materials was first reported in 1999.⁴ Furthermore, nanodimensional bioceramics have been proved to possess higher bioactive properties due to their larger specific surface area, dissolving more rapidly and accelerating the rate of formation and growth of the HA layer on the glass surface if compared with micro-sized and bulk bioceramics of the same composition.³⁷⁶ Experimental studies have also shown that melt-derived multicomponent Co- and Sr-co-doped silicate BG particles with a finer size (9 µm) possess a more effective osteogenic potential and higher cytotoxicity against Saos-2 cells as compared with larger particles (725 µm), due to their higher surface area, associated with higher ion release.

In vivo studies and clinical applications

The first reported clinical application of bioactive glass was the treatment of conductive hearing loss, with the reconstruction of the bony ossicular chain of the middle ear.³⁷⁷ Nowadays, bioactive glasses are applied for the regeneration of hard tissues, such as bone⁵⁹ and teeth.³⁷⁸ So, their main application fields remain orthopedic and dentistry, with several clinical commercial products to treat diseases in orthopedy and dentistry.¹¹³ However, BGs have also potential applications in cartilage regeneration,¹⁵ wound healing,¹³¹ nerve regeneration,³⁷⁹ and cancer therapeutics^{142,143} and products for the delivery of therapeutic dosages of radiation for cancer treatment, such as TheraSphere™, have been approved for clinical use by global regulatory agencies.¹¹³ On the basis of the author's knowledge, nowadays no formulation of drug-loaded BGs and bioactive glass-ceramics is already available on the market.

BGs (in detail, Bioglass® Ossicular Reconstruction Prosthesis, also known as Middle Ear Prosthesis, or MEP®) were first approved in 1985 by the FDA and then by several other international agencies.¹¹³ The MEP® gave its success to its ability to bond with both hard bone and softer tissues such as the eardrum and its manufacturing process using small molds, that allow the fabrication of monoliths with precise

dimensions based on the specific needs of each patient. Nowadays, it is not commercially available anymore, because of the dissolution and fragmentation of the implant.¹¹³

In orthopedics and maxillofacial surgery, 45S5 Bioglass® was firstly clinically used and is still applied as a synthetic bone graft material under two different product names, which are still available on the market: PerioGlas® and NovaBone®, both in particulate form.^{113,377} Nowadays, other commercial products based on 45S5 Bioglass® are available, such as Glassbone®, Vitoss® BA,¹¹³ and SIGNAFUSE®.¹¹³

In dentistry, BGs were firstly used as bone substitutes in dentoalveolar and maxillofacial reconstruction, periodontal regeneration, and implants, using commercial products like Unigraft®, and the already abovementioned PerioGlas®, Novabone® and Glassbone®,^{113,377} but many other applications are now available in the dental field.³⁷⁷ For example, BGs can be used for the fabrication of toothpaste as a hypersensitivity inhibitor (like NovaMin®, based on the 45S5 Bioglass® composition) and dental adhesives made of resin bonding systems containing BG fillers, such as micro-fillers of 45S5 Bioglass® or a Zn-polycarboxylated BG. BGs can be also used for the fabrication of coatings on dental implants;³⁷⁷ in this context, the addition of therapeutic and/or antibacterial doping ions is of great benefit. Besides the therapeutic and antibacterial effects of the released ions, the pH increase caused by the ion release from BGs can be also beneficial, exploiting a topical endodontic disinfectant effect with no negative impact on dentin stability.³⁷⁷

The first commercialized BG to be fabricated from a composition different from 45S5 Bioglass® was S53P4 (BonAlive® Biomaterials), a silicate-BG with the composition of 53% SiO₂, 4% P₂O₅, 23% Na₂O, and 20% CaO (in wt%) and a slower dissolution rate than 45S5 Bioglass®, produced in form of granules and a putty system, for the treatment of bone infections and diabetic foot osteomyelitis, and use in spinal fusions.¹¹³

In the field of wound dressing and infection fight, several biomedical products based on BGs are already available. An interesting example is the antibacterial Ag-doped resorbable phosphate BG particles Arglaes®, developed by Maersk Medical in the UK and marketed by Medline Industries (since the late 1990s), which can be combined either with a polymeric adhesive or with alginate powder for wound dressing, burn care, topical medication and prolonged controlled antibacterial effects (thanks to the slow release of Ag ions).²¹⁵

A doped BG (named Vitryxx®), based on the composition of the 45S5 Bioglass®, is applied in cosmetics for its anti-odor and anti-oxidant properties and its ability to reduce the visibility of wrinkles.²¹⁵

As anticipated in the "Introduction", one of the main drawbacks of the clinical applications of implanted BGs is the development of infection. A systematic review of the clinical applications of the bioactive glass S53P4 in bone infections was published by Bigoni *et al.* in 2019.³⁸⁰

Besides their incredible therapeutic and antibacterial properties, BGs possess some other properties like *in vivo* degradation,³⁸¹ ion release,³⁸¹ devitrification,³⁸¹ and brittleness,



To monitor the long-term effects, performance, and safety of device usage among the general population, data are still collected.¹¹³ For example, the FDA MedWatch program allows both healthcare professionals and the public to report adverse effects of medical devices and drugs that they have experienced.¹¹³

It is clear that novel micro- and nanostructured surfaces can overcome the limitations of ion- and drug-releasing systems. For example, antibacterial biomimetic nanostructured surfaces can overcome the limits of antibacterial release-based systems, such as particles containing or scaffolds and coating releasing antibiotics and antibacterial ions,²⁴ for example, BGs

Table 4 Tailoring strategies and their effect on cells

Type of cells	Method	Magnitude order of modification	Effect on cells	Advantages	Disadvantages
Eukaryotic cells	Ion incorporation (doping)	Macro	Depends on the added ions (angiogenic or osteogenic, and so on...)	Possibility to modulate cell response by simply varying glass composition Influence even on cells that are not in direct contact with the material, various simple methods of doping a bioactive ceramic	Dose-dependent response Need for replenishment or replacement
	Functionalization	Macro	Depends on the linked moieties (angiogenic or osteogenic, and so on...)	Various simple methods of functionalizing a bioactive ceramic Easy variations of material surface When the linked moieties are released from the surface, an influence even on cells that are not in direct contact with the material	Dose-dependent response Need for replenishment or replacement
	Topological modification through deposition methods	Micro or nano	Stimulation of adhesion, differentiation, and proliferation (through mechano-transduction of external signals)	Most eukaryotic cells are anchorage-dependent	Influence only on cells in direct contact with the material surface
Bacterial cells	Ion incorporation (doping)	Macro	Induction of apoptosis of bacterial cells by hindering some of their metabolic pathways	Various simple methods of doping a bioactive ceramic	Dose-dependent Burst-release kinetics Sublethal background dose Impossibility of killing attached bacterial cells Need for replenishment or replacement
	Functionalization	Macro	Induction of apoptosis of bacterial cells (if antibacterial ions are released) OR anti-fouling behavior	Various simple methods of functionalizing a bioactive ceramic Easy variations of material surface	Impossibility of killing attached bacterial cells Need for replenishment or replacement
	Topological modification	Micro or nano	Contact-killing, anti-adhesive, or entrapment strategies	Ability to kill bacteria that adhere to the surface	If bacteria do not enter into contact with the material, they cannot be killed Still not complete understanding of the mechanisms by which topography influences bacterial behavior Unclear over the influence of dead bacterial cells that remained on the material surface after being killed Variable response depending on the bacterial cell, so that the antibacterial surface must be designed <i>ad hoc</i> for the target bacterial strain

doped with antibacterial ions like silver, copper, cerium, or zinc. The main drawbacks of the released-based systems are burst-release kinetics that can make the compound ineffective, insufficient concentrations that reach the target (since diffusion is uncontrolled and directionless), a sublethal background dose that can trigger the development of resistance mechanisms, environmental aspects, and a need for replenishment or replacement.^{26,317} Moreover, these materials do not

kill bacterial cells that manage to attach to the surface, so these bacteria can still grow and produce biofilms.²⁶ All these problems could be solved by using antibacterial non-releasing nanostructured devices that can kill bacteria without the release of any chemicals or biocides, but only thanks to the contact between the antibacterial materials and the bacteria.

On the other hand, the advantage of physically-modified patterned surfaces is also their limit, because they can exploit



Regarding the chemical approach based on functionalization and coating, they are versatile tools for the modification of the outermost surface layer of biomaterials, allowing a tailoring of the interaction of the implanted materials with the biological environment (which mainly occurs at the interface surface), without any alteration of the bulk properties of the biomaterials.²⁸⁴ In this way, it is possible to combine the starting features of the biomaterial with the ones added at the surface.²⁸⁴ However, possible further drawbacks of these strategies could be the issues related to the long-term stability of the functionalization/coating,²⁵¹ possible variations of the bioactivity rates,^{247,389} and an unwanted physical modification, associated with the deposition of functionalizing molecules and metallic *in situ* synthesized nanoparticles, and related toxicity.³⁹⁰ In addition, the deposition of BG and bioactive glass-ceramic coatings can alter the wettability of the implant;³⁹¹ this wettability modification should be taken into consideration when planning *in vivo* applications, since it is well-known that the hydrophilicity of an implanted material influ-

ences the attachment of cells, both eukaryotic mammalian and bacterial cells. Moreover, a reliable comparison of the performance of different coatings applied on non-flat substrates (like realistic implants) is not easy, because very often there are no standard methods or procedures for their characterization, like in the case of the measurement of the adhesion strength of the coating to the substrate (in this case, only international recommendations for testing flat samples are available).²⁵¹

As discussed in depth in the section “Influence of topography on cell behaviour – surface and internal nanostructure”, another important limit of physically-modified nano- and microstructured bioactive glasses is the difficulty in fabricating them with the desired *ad hoc* nano- and microfeatures. Besides technological limitations, the mechanisms regulating the response of mammalian and bacterial cells are still not completely understood and literature information about parameters affecting this response is controversial.³²⁷ Taking into consideration the promise of these modified surfaces for TE and antibacterial applications, researchers should continue their investigation of both suitable fabrication methods and different surface parameters affecting the cell response. In this context, the development of standardized biological tests could be of great benefit, helping in the critical comparison of different nano- and microstructured surfaces.³⁹²

Regarding BG nanoparticles and their interactions with cells, the advantages given by their small size (such as a higher surface area, higher bioactivity, faster degradation, and quicker release of therapeutic ions) come immediately to mind;¹¹³ however possible issues related to cytotoxicity caused by their small size and ion release should be properly investigated.

In addition, both in the case of chemically- and physically-modified surfaces, the effects of these modifications on bioactivity and ion release are still under investigation, even though, according to a previous review by Kargozar *et al.*,²⁸⁴ published in 2019, bioactivity is generally maintained after BG surface functionalization.

In addition, the immobilization of biomolecules, such as drugs and growth factors, is complex, unstable, and more expensive, in comparison with ion doping, which is a relatively simple, stable, and low-cost approach.¹³⁵

Regarding the strategy of doping with ions, up to now, almost all elements of the periodic table have been added to a glass composition. So, the new frontiers of doping are the synthesis of co-doped BGs and the study of the synergic effects of multiple doping ions. As previously discussed, several experimental studies of co-doped BGs are already available, but a comprehensive understanding of their synergic effects is still lacking.

For all these strategies, the modulation of the glass reactivity is a further open issue, because a high surface reactivity can render chemical treatments and etchings more difficult and cause both a too quick release of doping ions, loaded drugs, and grafted molecules and a rapid change of the modified surface, making the chemical and physical modification of the surface ineffective.

To solve all the abovementioned open and challenging issues related to the here-reviewed tailoring strategies and reduce the experimental time, costs, and ethical issues, the synergical implementation of computational strategies, such as computational simulations and machine-learning, could be beneficial.^{22,393}

To meet the rising demand for customized BG-based substitutes, 3D processing technologies are needed;¹³ they can be used for the fabrication of tridimensional scaffolds with a hierarchical porosity and could also potentially help in the fabrication of BG devices with nano- and microfeatures. However, the 3D additive processing of ceramics still suffers from some limitations, such as:

- the possibility of air-bubble entrapment in the ink (in the case of robocasting),³⁷⁰
- suboptimal sintering of the ceramic powders in the case of the direct selective laser sintering (SLS) of ceramic parts,³⁹⁴
- reduced resolution, possibility of printing process imprecision, uneven distribution of the filler particles inside the printed structure (resulting from poor dispersion and sedimentation of the ceramic powders), and a limit of ceramic filler concentration equal to 40%, in the case of stereolithography (SLA),³⁹⁵
- difficult control over materials' properties at the nano-scale (whereas macro- and microfeatures can be produced with a good degree of control),³⁹⁶
- shrinkage³⁷⁰ and long-lasting optimization of the process parameters³⁹⁷ in general,

leading to unwanted inner voids and cracking in the case of the filaments of robo-casted scaffolds,³⁷⁰ poor morphological and mechanical properties in the case of direct SLS and SLA,^{394,395} and difficulties in the fabrication of nanostructured materials.³⁹⁶ The realization of nano- and microfeatures using 3D printing and other additive manufacturing processes is, indeed, still an open challenge.³⁹⁸ In any case, these methodologies allow a good control of dimensions, macro- and microstructural features, and porosity (*e.g.* pores as small as the ones in the trabecular bone can be obtained).^{370,399,400} In addition, it is interesting to point out that some promising composite microspheres and composite porous scaffolds, containing bioceramics, have been already successfully fabricated, overcoming some of the above-reported limitations of additive techniques.^{401–405} To fabricate nano- and microfeatured bone scaffolds, a well-defined microfeatured polylactic acid (PLA) scaffold was printed and nano-HA was then conjugated on its surface through post-processing aminolysis.⁴⁰⁶ Composite patches with multiscale 3D porosity were produced by combining extrusion 3D printing and electrospinning.⁴⁰⁷ In the actual review, we will not add further details, because a detailed discussion about the advantages and open issues of the additive 3D processing of bioceramics is out of the scope of the actual paper, but more info is available in the following reviews.^{395,408}

Making a general final consideration about BGs, independently from the used tailoring strategy, in the case of the emerging field of soft TE, in particular for wound healing, the com-



plete dissolution of the BG device and the absence of apatite-forming ability are desirable, allowing the wound site to fully heal with the host tissue, without mineralization of the healing tissues.¹¹³ Besides potentially altering the healing soft tissue and its mechanical properties (making it more rigid), HA formation could inhibit hemostasis, and calcium deposits could delay or even stop the healing of leg ulcers.¹¹³ Thus, the application of soft TE seems to be in contrast with the inherent and typical bioactivity of BGs.¹¹³ The use of BGs for these specific applications is highly challenging, but new BG compositions, that do not lead to the formation of a calcium phosphate layer on the material surface, are actually investigated.¹¹³ In this regard, the term “bioactive” is not referred anymore to the formation of hydroxyapatite but, rather, to the stimulation of a beneficial biological response through ion release.¹¹³

In this context, novel polymer composite scaffolds and hybrid materials containing doped BGs are showing promising results in both hard and soft TE. Another emerging application in which hybrid BGs are gaining increasing interest is cartilage repair and regeneration.¹¹³

According to the target application, the connectivity of the BG network, and ultimately the extent of apatite-forming ability, can be properly tailored by adding the proper amount of specific ions,²² as discussed in depth in “Doping with ions and loading with drugs – Bioactive properties”. Indeed, as previously written, while the bioactivity is desirable for bone TE, it is not advisable in contact with soft tissues (e.g., dermal regeneration).

We can conclude that, nowadays, each of the discussed tailoring strategies offers different advantages and disadvantages, on the basis of the intended final use.

The best modulation strategies of the glass properties are still an open issue and multidisciplinary investigation approaches are running to contribute to solving this issue.

In addition, to ensure reproducibility and successful scalability and translation to industrial market and *in vivo* applications, the standardization of the fabrication procedures is necessary.²³⁰

Author contributions

Piatti Elisa: writing – original draft; Miola Marta: writing – original draft, supervision; Verné Enrica: writing – original draft, supervision; conceptualization.

Conflicts of interest

There are no conflicts to declare.

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