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Optimized Bioactive Glass: the Quest for the Bony Graft

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Technological advances have provided surgeons with a wide range of biomaterials. Yet improvements are still to be made, especially for bone defects treatment. Biomaterial scaffolds represent a promising alternative to autologous bone grafts but in spite of the numerous studies carried out on this subject, no biomaterial scaffold is yet satisfying. Bioactive glass (BAG) presents many qualifying characteristics but they are brittle and their combination with a plastic polymer appears essential to overcome this drawback. Recent advances have allowed the synthesis of organic-inorganic hybrid scaffolds combining the bioactive properties of BAG and the plastic characteristics of polymers. Such biomaterials can now be obtained at room temperature allowing organic doping of the glass/polymer network for a homogeneous delivery of the doping agent. Despite these new avenues, further studies are required to highlight the biological properties of these materials and particularly their behavior once implanted *in vivo*. This review focuses on BAG

with a particular interest in their combination with polymers to form organic-inorganic hybrids for the design of innovative graft strategies.

1. Introduction

The human body possesses natural healing mechanisms to repair bone defects, but in many cases, these mechanisms are not efficient enough and endogenous repair has to be boosted. Bone loss affects more than 20 million people worldwide each year^[1] including 500 000 bone surgical procedures in the United States with a cost of over \$2.5 billion.^[2] Currently, most of grafting procedures involve autologous bone graft (receiver and donor are the same person). However, the quantity/quality of available tissue can be limited and the multiplication of surgical sites increases morbidity and can lead to complications at the donor site.^[3,4] Hematoma, bleeding, infections and chronic pain are frequently observed.^[5-7] Allografts (the grafts come from another individual of the same species) and xenografts (the grafts come from an individual of another species) are common alternatives.^[4,8-10] These grafts have some similarities with physiological bone with regard to its mineral part. However, limited osteoinductive properties and the inadequate stock in allograft biobanks presents a huge drawback^[11] which is caused by a significant cost for the collection and the storage of the tissues. Safety issues of these materials also remain a major concern although screening for diseases or infections have been improved.^[12-14] From a regenerative point of view, allo- and xenografts have more chances to be rejected and osseointegration is slower due to the treatments employed to avoid immunogenicity and the transmission of diseases/infections.^[15] As reviewed by Shibuya in 2015^[15], allo- and xenografts are as efficient as autologous graft for defects in well-vascularized areas^[16,17], however, incorporation can be difficult in less vascularized areas.^[18,19] Tissue engineering has been developed to overcome these limitations. The field has grown exponentially in the last twenty years and different types of synthetic biomaterials may be used instead of allo- or xenografts when autologous bone is not sufficiently available or not adapted to a particular need.

A large panel of biomaterials is already available in different shapes and properties.^[20] They can be inert (nearly inert) or bioactive, resorbable/degradable or permanent. Inert biomaterials present a minimal tissue response while bioactive materials are able to interact with host tissues.

Besides the biological behavior of the material, the determination of mechanical properties is an important aspect concerning the finding of an appropriate application. In 1678, Robert Hooke formulates: “*ut tensio sic vis*” that means “such expansion, such force” and that would be the basis of the characterization of material mechanical properties. This sentence explains the elastic behavior of most of the solids when they are submitted to a force. Most of the mechanical properties such as elastic modulus, yield strength, ultimate strength, ductility and toughness ensue from tensile or compressive tests. Fatigue tests complete the characterization.^[21] Generally, the Young’s modulus of the material should not exceed the Young’s modulus of the bone to which the material tends to bond. The Young’s modulus of human bones is highly correlated with bone density and depends on a wide range of parameters including the age, the type of bone or bone health status. For example, the Young’s modulus of cancellous bone can vary over ten folds depending on the localization of the bone in a healthy people.^[22] If this mechanical aspect is neglected, stress shielding of the bone tissue may lead to a reduction of the bone density. Besides, plastic properties are usually required to avoid brittleness and subsequent instability of a material that is subjected to cyclic mechanical stresses.

Depending on the characteristics of the bone defect and the patient’s conditions, different approaches may be considered. Particulate systems are often used by the surgeon. For example, synthetic particles of different shapes and sizes may be mixed with patient blood and autologous bone to form a putty-like material that can be easily pressed into the bone defect.^[23] Injectable materials with bioactive particles embedded in an injectable matrix are also considered for the administration of materials directly inside bone defects. The major flaw of particulate systems is their poor stability when used in unconfined defects for which external fixation is needed. The degradation rates of the two components can also be an issue.

Unlike monolithic biomaterials, scaffolds are porous and can be colonized by cells and blood vessels. Among biomaterials, the class of bioactive glasses (BAG) presents advantageous features for a utilization as scaffold.

The present work will not systematically review the commonly used biomaterials but rather focus on BAG and how recent discoveries regarding their synthesis and their association with other materials could lead to innovative strategies in the field.

2. The Scaffold strategy

Nowadays, the approach of tissue engineering usually based on the utilization of scaffolds is considered as one of the best alternative in the treatment of osseous defects.^[24] An ideal scaffold is a temporary three-dimensional porous template that should stimulate bone regrowth until its complete replacement by new tissue.^[25] To match with this definition, biomaterial scaffolds should be **biocompatible, osteoconductive, osteoinductive, osteogenic, angiogenic** and **resorbable** or **degradable**. As for all biomaterials, they should exhibit mechanical properties adapted to their application.^[2,26–29]

The term **biocompatibility** have been introduce by Alexis Carrel, a French scientist laureate of the Nobel Prize in Physiology and Medicine in 1912. He described for the first time the different factors behind graft rejection (edema, inflammation and necrosis) which represent the basis of biocompatibility.^[30] The term **osteoconduction** have been defined at the consensus conference of the European Society for Biomaterials in Chester in 1987. It refers to the capacity of a material to allow bone growth on its surface.^[31] **Osteogenesis** is the production of physiological bone tissue through the recruitment and the activity of osteoblasts, it is mandatory for bone defects healing. An **osteoinductive** biomaterial is able to stimulate the differentiation and the activity of bone cells (osteoblasts, osteoclasts and osteocytes) in order to promote osteogenesis.^[31]

Degradable/resorbable scaffolds are designed to be replaced by bone tissue and degradation products must not be toxic. The term “**biodegradation**” is employed when materials degrade under

the control of biological agents (enzymes). “**Bioerosion**” is used when degradation occurs solely under physical stress (e.g. water solubility). Finally, “**bioabsorption**” and “**bioresorbability**” are used when the products of the material’s degradation are eliminated or used by cells.^[31]

Scaffold biomaterials structure should be rapidly vascularized in order to bring new cell precursors and to achieve a sufficient supply in nutrients and oxygen. Therefore, the colonization by vascular precursors follow by their differentiation and the establishment of a rapid vascularization system is critical.^[2,26] For this purpose, an interconnected porosity is required.^[32,33] Porosity greater than 50% is intended to allow, fluid circulation, cellular invasion and vascularization. According to cell size, macropores with a minimal diameter around 100 μm are required but diameters over 300 μm are recommended for a better vascularization.^[34] Pore chambers must be interconnected to avoid dead spaces with interconnection windows of 100 μm .^[35,36] Designing highly porous scaffolds with adapted mechanical properties represents a major challenge in bone tissue engineering. Besides, biomaterials endowing delivery systems of agents to enhance the aforementioned characteristics would represent a competitive advantage.

3. Glasses

3.1. History

The “ceramic” term comes from the Greek word “*keramos*” which means “burned soil” and is a reference to pottery and to the thermic treatment commonly used in the field. From a biomaterial engineering view point, ceramics present interesting characteristics including hardness, mechanical strength and stiffness. Furthermore, ceramics can be biodegradable and their dissolution products may be used by surrounding cells without toxic effects.^[37] The most common ceramics used today are hydroxyapatite (HaP) and calcium phosphate (CaP) that are very close to the mineral phase of the bone matrix in term of structure and chemical composition. Those materials are known to be osteoconductive and to form an interface with bone without forming scar tissues.^[38-40] Such ceramics have been extensively used for filling defects (cement, granules) and for the coating of

metallic materials.^[41] Ceramics particles can be assembled to obtain macroporous blocks but they result in a poorly interconnected porosity and lead to a difficult circulation of fluids. Moreover, the brittleness of ceramics limit the applicability of such porous biomaterial.^[42] As an example, the comparison between biphasic calcium phosphate particles and porous blocks made by sintering these particles (1100°C) and implanted in rat calvarial defects shows a limited bone formation inside the blocks and a better reconstruction in the particulate systems.^[43] This weak bone reconstruction is thought to be associated with an irregular porosity and a poor pore interconnection inherent in the technique of blocks production.

Among ceramic family, other members may also be relevant for bone materials especially BAG.^[44-46] Glass has been discovered more than 5000 years ago in the region of Syria according to the Roman historian *Pliny the Elder* (23-79 CE). It has only recently appeared in the field of medicine. The first medical glass has been invented by Pr. Larry Hench at the University of Florida in the late sixties.

After the Vietnam war, many wounded people needed proper medical care and effective biomaterial for bone trauma repair were not available. At that time, most of the biomaterials were considered as inert (metallic implants or polymers) regarding the approaches for biological behavior of those compounds at the time and concepts of biocompatibility in this period. Following a discussion with a US colonel, Larry Hench started searching for a better biocompatible material.^[47] In 1969, he achieved the synthesis of a degradable bioactive glass (BAG) able to bond to living tissue^[48-51] and particularly to bone. The original Hench 45S5 BAG latterly trademarked Bioglass[®] has been involved in the repair of bone defects for more than one million patients.^[52] This discovery paved the way to the development of many bioactive materials in the field of bioactive ceramics including variants of the original 45S5 Bioglass[®], glass-ceramics and other ceramics such as HaP and CaP.^[50] For instance, a clinical trial involving S53P4 BAG in the treatment of non-union of the tibia and femur in comparison with autologous/tricalciumphosphate graft have been approved and recruitment is in progress.^[53]

To date, the family of bioactive glass is composed of different members including silica-based BAG such as the 45S5, phosphate-based bioactive glasses and borate-based bioactive glasses.

3.2. Synthesis routes at a glance

Hench's BAG was prepared by melt quenching route and was called 45S5 (46.1 mol.% SiO₂, 24.4 mol.% Na₂O, 26.9 mol.% CaO and 2.6 mol.% P₂O₅) or Bioglass®. Briefly, oxides (Na₂O-CaO-SiO₂-P₂O₅) are melted in a platinum crucible and quenched in a graphite mold (for rods and monoliths) or in water (frit).^[47] This method involves high temperature treatments (above 1300°C). In the 90s, the advent of the sol-gel soft chemistry route allowed the production of glasses at lower temperature than those involved in melting methods. Furthermore, this technique allowed the simplification of bioactive glasses to ternary (SiO₂-CaO-P₂O₅) and binary (SiO₂-CaO) systems.^[54] Briefly, this route takes place in three stages: the preparation of a sol, its gelation and the evaporation of the solvent.^[54] A sol is a suspension of colloidal oligomeric precursor species (1 – 1000 nm) that will progressively condense until a continuous 3-D macromolecular network is obtained, generating a gel at room temperature.^[55,56]

3.3. Structure and composition relationships

The main component of most silica-based BAG is silicon dioxide (SiO₂). It forms the basic building unit of the glasses via its siloxane bonds (Si-O-Si), where a bridging oxygen connects two silicon centers. Network modifiers which are alkali or alkaline-earth metals (generally Ca²⁺ or Na⁺), disrupt the tetrahedron network by creating non-bridging oxygen atoms such as Si-O⁻ M⁺ (M⁺ being a modifier cation). This is an important aspect of BAG, since an open glass structure is required for bioactivity. By varying the composition and the amount of network modifiers, many synthesis parameters can be set, including transition temperature, softening point and solubility^[57] (**Figure 1**). The notation Qⁿ is used to describe the connectivity between silica tetrahedrons, where n represents the number of bridging oxygen atoms associated with the silicon. A ²⁹Si solid state magic-angle

spinning nuclear magnetic resonance (^{29}Si MAS NMR) study of Bioglass[®] suggests that 69% of the silica network consist of Q^2 silica tetrahedrons in form of chains and rings, whereas 31% are Q^3 units, contributing to increased cross-linking.^[58] An increase in Si content causes a higher proportion of Q^3 silica, which could be shown by comparing different glass compositions.^[57] In 1995, West and Hench already tried to model the lowest energetic ring size in amorphous silica since there is no technique to determine experimentally their geometry in this system. However, the different models predicted either the 4-membered or the 5-membered ring to be the most stable.^[59] ^{17}O MAS NMR has also evolved as a useful technique to study the order of a silica network since it is able to distinguish bridging and non-bridging oxygen atoms.^[60]

Finding the right balance between bridging and non-bridging oxygen atoms in bioactive glasses is crucial in order to maintain the adequate mechanical properties of the biomaterial and obtain the desired degradation behavior while maintaining satisfying biological properties. A study on the incorporation of calcium into the silica network in sol-gel BAG is particularly relevant to give an overview of the importance of network modifiers in the network connectivity.^[61] In this study, calcium silicate gel heated up to 350°C shows a dominant silica network with bridging oxygen atoms around which calcium highlight that salts probably form a layer. At higher temperatures above 500°C , calcium migrates into the silica network as indicated by an increasing number of non-bridging oxygen atoms.^[61] According to a molecular dynamics (MD) simulation performed by Tilocca's group^[62] on Bioglass[®], clustering of calcium has been observed with calcium enriched regions containing CaO structural patterns.^[58] Ca^{2+} rather associates with a phosphorous environment than with the silicate network.^[63] Hence, it is more likely that Ca^{2+} interacts with the non-bridging oxygen atoms of the silica in the absence of phosphate, decreasing the network connectivity. Consistently, calcium released by biomaterials creates a supersaturation that enhances apatite precipitation.^[64,65]

Thus, phosphate (P_2O_5), present as orthophosphate (PO_4^{3-}), is often used as a second network former.^[57,58,63] Calcium and sodium act as charge balancers for the phosphate anions and are

prevented from their network-modifying role.^[63] The reason may lie in their similar ionic radii.^[66] Next to the interaction with calcium and sodium, it was debated whether phosphate interacts with silica and forms labile Si-O-P bridges. *In-situ* Fourier-Transform-Infrared spectroscopy suggests that these bonds are formed but dissociated easily in water.^[67] However, this hypothesis was no longer supported by nuclear magnetic resonance (NMR) and optical data.^[58] The absence of Si-O-P bridges in 45S5 Bioglass was confirmed by another ³¹P NMR and ¹⁷O multi-quantum magic-angle spinning (MQMAS) experiment.^[68] Another phosphate species, pyrophosphate (P₂O₇⁴⁻), could be detected in small amounts in some phosphosilicate bioactive glasses.^[58,63] The role of this different phosphate form remains under debate, especially concerning the bioactivity of BAG.

3.4. The biological activity of Silicate based Bioactive Glasses

In the field of biomaterials, the term “biological properties” gathers all the phenomena related to the study of a biomaterial in the presence of biological material. Bioactive glasses were initially designed to replace materials considered at that time as inert in bone defect treatments and investigations on their biological properties reinforced their potential for regenerative medicine.

To consider the biological properties of a bone biomaterial at a physiological level, one must be aware of the biological mechanisms responsible for the formation of bone tissue. At a glance, bone formation is a complex process based on the sequential activities of different bone cell types. Osteoblast cells evolve from mesenchymal progenitors and are involved in bone matrix deposition and mineralization.^[69] On the other hand, osteoclasts evolve from hematopoietic precursors and are responsible for bone resorption. The differentiation of these two cell types is regulated by different control factors according to the needs of the organism. The transcription factors runt-related transcription factor 2 (Runx2) and osterix (Osx) are essential for osteoblast differentiation.^[70] During the final stages of differentiation and activity, an osteoblast can undergo apoptosis or be embedded into the mineralized matrix to act as a regulator of mineral metabolism by coordinating the activities of osteoblasts and osteoclasts in response to mechanical stimuli.^[71] Osteoclast

differentiation involves both macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor-kappa B (RANK) ligand (RANKL). RANK/RANKL interaction activates Nf-kB pathway and initiates the fusion of osteoclast precursor cells, leading to the formation of functional multinucleated osteoclasts.^[72]

In a context of a regenerative medicine supported by a biomaterial, inflammation plays a critical role in the success of the graft. Indeed, the surgical procedure will cause bleeding and therefore a hematoma that will trigger an inflammation process. This phenomenon is essential for healing as it enhances new bone formation through the recruitment of osteoblast and endothelial precursors^[73]. On the other hand, the participation of biomaterials in this biological process should be limited since an excessive inflammation can have detrimental effects.

Once implanted and without the need of bone cells, bioactive glasses have the ability to rapidly bond to bone.^[74] Interestingly, these glasses are able to make a strong and stable interface with living bone tissues and to enhance bone formation without showing cytotoxicity.^[37,74-77] The basis of bone-bonding consists in an initial surface dissolution of bioactive glass leading to the formation of a hydroxyl carbonate apatite layer (HCA). This layer is similar to natural bone and bridges the bioactive glass and the bone tissue.^[50] To shed light on this process, a complex five-stage reaction has been proposed by Hench and West in 1996.^[78]

Additionally, BAG possesses osteoinductive properties. For instance, 45S5 Bioglass® induces osteoblastic activity due to both, apatite crystallization at its surface and the release of its ions. This induction is even greater than hydroxyapatite itself.^[79] The dissolution products (Si, Na, Ca, phosphate ion, etc.) are known to stimulate bone formation^[39,80] (**Figure 2**). These data are supported by *in vivo* studies^[81-83] showing that bone repair with 45S5 Bioglass® is more efficient than any other kind of bioactive ceramics. This is thought to be triggered by the overexpression of several gene families in human primary osteoblasts. Among them, there are genes that encode for extra-cellular matrix remodeling proteins (e.g., metalloproteinases), for transcription factors (e.g., c-

myc, PuF) or cell cycle regulators.^[84–86] Silver et al. showed that 45S5 dissolution products induce an alkalization of the osteoblast micro-environment leading to glycolysis, cellular ATP production and changes in the proton concentration.^[87] They showed that the presence of 45S5 dissolution products increases the calcium concentration in cells with a potential impact on intracellular enzymes and metabolic pathways. Furthermore, the presence of calcium in a monolayer and 3-D culture of primary osteoblasts has been shown to be important for cell survival, proliferation, differentiation and for matrix mineralization through the upregulation of IGF–II and the production of glutamate.^[88–90]

Besides the stimulation of bone formation, bioactive glasses possess interesting antimicrobial properties. Generally, the implantation of a foreign body induces a risk of infection leading to poor tissue integration and even sepsis.^[91] Interestingly, BAG are proven to clearly inhibit the growth of a wide selection of bacterial species known to be responsible for clinical infections and graft failure.^[92,93] The antibacterial effects of bioactive glasses seem to rely on the increase of pH at the vicinity of the BAG.^[94] In a clinical study, the use of NovaBone®, a trademark of the 45S5 BAG, was compared to autografts in posterior spinal fusion operations for the treatment of adolescent idiopathic scoliosis (curvature of the spine) in 88 patients. Consistent with the aforementioned data on BAG, this biomaterial performed as well as autograft over 4 years with fewer infections.^[95] A variant, the BAG-S53P4 (53% SiO₂, 23% Na₂O, 20% CaO and 4% P₂O₅ (wt) - BonAlive®), was tested in 27 patients affected by chronic osteomyelitis in long bones, an infectious process that can lead to bone loss for the most severe cases. After a 36-month-follow-up period, results show that the patients treated with BAG and without local antibiotics achieved similar eradication of infection and less drainage than those treated with two different antibiotic-loaded calcium-based bone substitutes.^[96] This anti-microbial activity relies on its composition, however, more randomized clinical trials need to be performed in order to determine whether bioactive glass would be able to replace the use of antibiotic in the case of osteomyelitis.^[97] More recently, the same authors reproduced these results in 116 multinational patients with a median age of 48 years old (15-87).

The majority of these patients have been implanted without the addition of local antibodies (85%) and the success rate was 90% after 1 years highlighting the possible use of BAG without further antibiotic medication.^[98] An overview of clinical trials on S53P4 bioactive glass in various situation has recently been given.^[97]

Finally, it is worth noting that BAG (45S5) present an inherent pro-angiogenic activity.^[99] BAG are able to stimulate the angiogenic response of human microvascular endothelial cell cultures^[100] and CCD18Co human fibroblasts^[101] through an up-regulation of the secretion of vascular endothelial growth factor (VEGF). This property is not limited to the 45S5 BAG since extract from 58S and 80S BAG are able to stimulate the proliferation and increase VEGF expression of human umbilical vein endothelial cells (HUVEC) leading to the development of vascular tubes *in vitro*.^[102] According to these results and a previous study, the release of calcium ions could be responsible for this effect.^[102,103]

3.5. Non-silicate based Bioactive Glasses

Next to conventional silicate bioactive glasses, including the 45S5 Bioglass[®], other glasses have been developed: phosphate- and borate-bioactive glasses.^[104] Their network formers are usually phosphorus pentoxide (P₂O₅) and boron trioxide (B₂O₃), respectively.^[105]

Degradation rates of silicate, borosilicate and borate bioactive glasses were compared.^[106–108] An increase in the B₂O₃ content improved the conversion to hydroxyapatite. Compared to Si, which has normally a coordination number of four, B₂O₃ is coordinated three times, leading to a decreased condensation of the three-dimensional glass network and a more rapid dissolution rate.^[108,109] *In vitro* borate glass scaffolds inhibit cell proliferation and functions in murine osteoblast/osteocyte-like cells (MLO-A5). This is thought to be related to the fast release of boron ions, which are toxic above a certain concentration. A recent study shows how the addition of strontium in borate-based glasses avoids the cytotoxic effect of boron *in vitro* and improves the adhesion of osteoblast-like cells on a material slice.^[110] However, *in vivo* a more dynamic fluid exchange reduces the local

boron concentration after implantation and uncovers the performance of the borate bioactive glass scaffolds.^[111] Furthermore borate glass dissolution drives boric acid production, an antiseptic that might explain the positive application of microfibrinous borate-based bioactive glass in the healing of chronic soft tissue wounds.^[112]

Similar to borate-based bioactive glasses, phosphate-based glasses have a low resistance to water attacks and thus dissolve faster. Despite phosphorus having a valence of five in phosphorus pentoxide, its maximum speciation is three due to one double-bonded non-bridging oxygen. This leads to a reduced cross-linked glass network.^[113] However, dissolution rates can be well-modified by the utilization of ternary glass systems such as $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5$ ^[114] or dopants, e.g. TiO_2 , with a high valence, stabilizing the glass network.^[115] *In vitro* tests with human pulp cells resulted in improved bioactivity in simulated body fluid (SBF) and decreased cytotoxicity when the calcium containing phases were increased.^[116] This ternary system doped with titanium further increases proliferation and expression of osteoblastic markers in human osteosarcoma cell line (MG63).^[115] Since the long-term effects of silicon ions are not completely known, phosphate glasses have retained a major consideration for bone tissue replacement. At the beginning of the development of BAG, phosphorus was thought to be essential for the phenomenon of bioactivity. But in the early 1990's, a P_2O_5 -free glass in a binary system (SiO_2 , CaO) was shown to be bioactive *in vitro* and *in vivo*.^[117] More recently, a binary system have been compared to melt-quenched glasses and ternary sol-gel glasses, according to these studies the three materials are comparable in terms of bioactivity and they present similar dissolution rates.^[55,118]

The biological activity of a biomaterial might be enhanced by the modulation of several parameters, including composition, heat treatment and synthesis route, influencing furthermore the shape and dissolution rate. However, as it is the case for other ceramics, bioactive glasses present a high brittleness, which leads to a poor fracture resistance when submitted to cyclic loads. Therefore,

alternative synthesis processes and compositions need to be developed for BAG in order to improve their medical relevance.

4. Inorganic – Organic couples

Since Hench's 45S5 Bioglass®, many ways have been investigated to improve the relevance of bioactive glasses for bone defects healing including the evolution of synthesis processes (sol-gel). However, as aforementioned, the main concern with BAG remains its brittleness. Thus, a major challenge for biomaterial engineering is now to provide surgeons with BAG presenting both adequate mechanical properties and enhanced biological properties. To address these issues, organic-inorganic associations have been developed. This session will discuss the most commonly used organic polymers and the type of association to further focus on hybrids especially sol-gel hybrids and how they could be considered as a great opportunity for innovative strategy designs.

4.1 Who is the best organic mate?

Polymers consist of repetitions of small units or monomer that form chains of various sizes and shapes with subsequent different chemical properties that are extensively used in the medical field. Different types of polymer can be associated to obtain copolymers with a wider range of properties.^[119,120] The first medical use of a temporary homopolymer dates back to the sixties with "Dexon" a biodegradable suture constituted of polyglycolic acid (PGA).^[121] Polymers are of particular interest for bone repair as their chemical properties may vary according to synthesis and process. The degradation rate of polyphosphazene can easily be set by modulating the nature of their side groups resulting in favorable osteoblast adhesion.^[122] Modifying polycarbonates with tyrosine-derived amino-acid increases their mechanical properties and their potential as biodegradable candidates^[123,124]. Linear aliphatic poly(α -hydroesters) such as poly- ϵ -caprolactone (PCL), polyglycolic acid (PGA), polylactide (PLA) and their copolymers such as poly(lactic-co-glycolic

acid) (PLGA) are the most popular synthetic materials used in medicine. These materials are approved by the Food and Drug Administration (FDA) for different applications in humans.^[125] These materials are biocompatible, induce minimum inflammatory response and are degraded by hydrolysis and enzymatic reactions into dissolution products handled by regular metabolic pathways.^[122,123,126] PLGA is known to be compatible with osteoblast migration and proliferation.^[122,127] Moreover, PLGA materials can be engineered to have the same degradation rate than bone ingrowth.^[127] However, such polymers are not suitable on their own, especially for load bearing bone treatment, due to their weak mechanical properties. Moreover, they do not present bioactive nor osteoconductive properties.^[128,129]

Besides synthetic polymers, natural polymers may be isolated from living organisms and treated (or not) to reduce a potential inflammatory response or pathogen/disease transmission. Among this family, the use of collagen makes sense as it represents more than one-third by weight of the body proteins.^[130] To date, 28 types of collagen are known, whereas type I collagen is the most represented. It can be found for example in bones, skin and tendons.^[131] Gelatins can be obtained from different collagen hydrolysis processes (acid, alkaline, thermal). This material forms scaffolds of lower antigenicity compared to untreated collagen. Collagen-based biomaterials can be enzymatically degraded by different proteases such as metalloproteinases and serine proteases. Collagen-based bone biomaterials are mainly used in the form of membranes in guided bone regeneration (GBR).^[132-134] Recently, dense collagen lattices have been processed, seeded with dental pulp stem cells and used in rat critical-size calvarial defect models. This study based on microcomputed tomography and histological analysis has highlighted the good potential of cell loading in such biomaterials.^[135]

However, the clinical demand seems to be more oriented towards easy-to-use biomaterials, which is not fulfilled by the addition of cells, making the implantation procedure more complex.

Chitosan is the most represented natural polymer. It can be obtained by deacetylation of chitin, a polysaccharide mostly extracted from the shell of crab, shrimp or crawfish by chemical hydrolysis.

As for collagen, chitosan of different molecular weight can be obtained depending on the method of production. This polymer is biocompatible, non-toxic, non-allergic and degradable making it a good candidate for tissue engineering applications.^[136] Thanks to its cationic nature, chitosan is able to make electrostatic complexes with negatively charged synthetic polymers.^[137] Different biological properties such as antitumor^[138,139], antimicrobial^[140] or antioxidant^[141] activities have been attributed to chitosan. Composite scaffolds made of gelatin-chitosan and calcium phosphates (HaP, β -TCP) or 58S bioactive glass particles have been produced by freeze drying and tested with human mesenchymal stem cells and in a rabbit tibia model.^[142] These biomaterials did not present the brittle characteristics of ceramics and enhanced cell proliferation and differentiation (RUNX2). Finally, these composite made of β -TCP or 58S bioactive glass presented a better bone regeneration *in vivo* when compared to HaP and negative control ; 58S bioactive glass being a slight more efficient than β -TCP. However, these composites lack of osteogenic properties and osteoconductivity as it could be supported by the association with a BAG matrix.

4.2. Composites and hybrids

A composite material combines at least two immiscible components in order to obtain new properties. In the case of BAG, such associations permit to obtain non-brittle bioactive porous scaffolds with tailorable mechanical properties.^[143] The main drawback of such material is the difficulty to obtain a homogeneous dispersion of the two components. Moreover, composites present apatite nucleation only at the BAG particles that protrude non-homogeneously from the surface. The poor exposition of the bioactive particles delays bonding to bone tissue *in vivo*^[144] and the heterogeneity causes instability and premature deterioration of the scaffold^[145] that makes composites poor candidates for bone engineering.^[25,146] To overcome these limitations, nanocomposites have been developed and seem to be of particular interest. Generally, nanocomposites consist of an organic matrix in which bioactive inorganic nanoparticles or nanostructures (<100 nm) are included. Different nanocomposites have been tested including

materials based on mesoporous silica particles.^[47,147] The main limitation of this type of biomaterial lies in the difficulty of incorporating the nanoparticles homogeneously in the polymer matrix. Moreover, the exposure and impact of glass nanoparticles on human health remain controversial and their possible toxic effects need further investigation.^[148–153]

Unlike composites or nanocomposites, sol-gel BAG-polymer hybrids are composed of inorganic and organic networks with chains entangled at a molecular level.^[154] The two components cannot be distinguished above the nanoscale and the material acts as a single phase, addressing the issue of homogeneity and improving mechanical properties when compared to the corresponding composite.^[155] Furthermore, hybrid materials are more capable to bond to bone because the whole surface of the material allows apatite nucleation. **(Table 1)**

Hybrids can be split into several categories: class-I hybrids in which weak interactions occur between the inorganic and organic networks (hydrogen bonds and van der Waals interactions) and class-II hybrids that are made of intricate matrices which are covalently linked by a coupling molecule.^[24] **(Figure 3)**

4.3. Sol-gel hybrids

The melt-quenching route to BAG is not compatible with the synthesis of organic-inorganic hybrids due to the low resistance of polymers to high temperatures. Room temperature sol-gel chemistry is compatible with the addition of a polymer to obtain a “hybrid sol”. Calcium is essential for the bioactivity of BAG and is known to trigger osteoblasts proliferation and differentiation.^[88] As aforementioned, calcium nitrates were initially used to incorporate calcium into the silicate network by heating. In the case of a hybrid, the presence of a polymer at an early stage of the sol-gel process forbids thermal treatments and calcium incorporation has become a real challenge.

The incorporation of calcium at ambient temperature can be achieved by using calcium alkoxide precursors instead of salts like CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ ^[156], but they are highly reactive towards water. Hence, the introduction of water needs to be limited to avoid a premature gelation of the BAG sol

that would make it harder to process scaffolds. Because of this restraint, many hybrid scaffolds presented in the literature do not contain calcium and possess a limited bioactivity^[155,157,158] or they do not incorporate calcium due to the use of salts.^[158,159] Only a few hybrids with well-incorporated calcium were obtained, but they were in the form of dense^[160,161] or fibre materials.^[162]

To avoid the restrictions that arise from the use of calcium, borophosphosilicate glasses $\text{SiO}_2\text{-B}_2\text{O}_3\text{-P}_2\text{O}_5$ (BPSGs) are a possible alternative to $\text{SiO}_2\text{-CaO-P}_2\text{O}_5$ and $\text{SiO}_2\text{-CaO}$ glasses. Indeed, boron is known to accelerate the degradation of BG and induce a faster apatite precipitation on its surface.^[107] This means that, even though BPSGs do not contain calcium, they have the potential to be bioactive. Mondal *et al.* developed a non-aqueous sol-gel process for the synthesis of BPSG-polycaprolactone hybrid scaffolds.^[163] The hybrid solution had a gelation time of 5 to 7 days depending on the organic content. The authors reported an apatite precipitation on the hybrid after 3 days of immersion in simulated body fluid. Although to our knowledge, a comparative study on the apatite-forming ability of BPSG and $\text{SiO}_2\text{-CaO}$ BAG has not been published yet, this work shows that BPSG-polymer hybrids are a possible alternative to calcium-containing BG-polymer hybrids.

Recently, Lao *et al.* developed a protocol that allows the synthesis of BAG-gelatin class-II hybrid scaffolds with $\text{Ca}(\text{OEt})_2$ as the calcium source.^[164] The gelation of the hybrid solution was delayed to 2 hours by using a reduced water-to-tetraethylorthosilicate molar ratio of two under dilute conditions. Connected pores within scaffolds were obtained using a porogen leaching method that involves neither heating nor the use of water. The scaffolds induced the formation of apatite within 3 days of soaking in simulated body fluid and had a compressive strength in the range of that of trabecular bone. This study demonstrates that hybrid scaffolds with well-incorporated calcium can be produced in a relatively simple manner. As illustrated in **figure 4**, these hybrids scaffolds present a highly interconnected porosity (**Figure 4**) and various forms can be obtained. Such process needs to be adapted for the synthesis of synthetic hybrids. This process also proved to be suitable for the synthesis of hybrid scaffolds comprising a synthetic polymer such as polycaprolactone.^[165]

Furthermore, properties of silicate-based hybrid materials based on sol-gel technique can be modulated by crosslinking between the organic and inorganic phases (Class-II hybrids). A key advantage of class-II hybrids is the potential for tailoring their degradation rates and their mechanical properties through the degree of covalent linkage. Crosslinking between the BG and the polymer chains can be achieved by employing an organoalkoxysilane-coupling molecule, *e.g.* glycidyoxypropyl-trimethoxysilane (GPTMS). The alkoxy silane functions link to BG by sol-gel reactions, while the organic group links to the polymer by nucleophilic addition (for example, the epoxide group of GPTMS reacts with the $-\text{COOH}$ or $-\text{NH}_2$ nucleophilic species of the polymer).^[166] Naturally-derived polymers like gelatin and chitosan contain many nucleophilic groups along their backbone chain, which allows a substantial crosslinking and therefore a great control over the properties of the corresponding hybrids.^[155,167] However, industrial demand seems to be shifting towards synthetic polymers because of easier regulatory approval. Synthetic polymers generally contain only one nucleophilic group at each end of their backbone chain, hence coupling with these polymers is very limited. The density of reactive species and therefore degree of coupling can be increased by reducing the molecular weight of the polymer^[160,168,169], but doing so the mechanical properties and degradation rate of the hybrid are greatly affected, thus negating the benefits of class-II hybrids. New synthetic polymers need to be developed to contain reactive species in their repeating unit. A promising approach consists in including monomeric units such as (trimethoxysilyl)propyl methacrylate (TMSPMA) in the radical polymerization of a chosen polymer.^[170,171] This results in a polymer containing pending alkoxy silane groups that allow covalent linkage with BG. In this case, the use of GPTMS (or another coupling agent) is not required. Compared to the post-functionalization of the polymer (*e.g.* with GPTMS), this technique allows a better integration of alkoxy silanes as part of the polymer and a greater control over the crosslinking density. Nevertheless, it is only applicable to polymers that can be synthesized by radical polymerization.

4.4. Interest of sol-gel route for 3D scaffolds

Many of the BAG are produced as particles or monoliths, but the weak relevance of particulate systems and monoliths in clinic has driven the idea that an ideal synthetic bone graft should be supported by a porous scaffold, a solid support for bone regeneration.^[24] Porous glass scaffolds can be obtained from 45S5 melt-derived particles. Briefly, particles are heated slightly below their fusion temperature in order to partially merge them, thus creating porous scaffolds. However, the difference in temperatures between partial fusion and crystallization is very narrow: partial crystallization is often obtained and reduces the BAG bioactivity.^[172] To limit crystallization, network modifiers such as K₂O or MgO may be used but they do not entirely address the issue. K₂O-containing BAG fibers can be processed. The S520 BAG (52.0 mol% SiO₂, 20.9 Na₂O, 7.1 K₂O, 18.0 CaO, and 2.0 P₂O₅) has been demonstrated to be able to support apatite-like formation after 5h soaking in SBF and favored primary human osteoblast attachment and mineralization after 14 days.^[173] However, the addition of other components increase the complexity of the structure and does not bring a fundamental advantage over partially crystallized 45S5 BAG scaffolds.^[174] Using sol-gel glass synthesis the silica network is assembled at lower temperatures^[175,176], thus preventing crystallization while simplifying composition (binary, ternary) and preserving bioactivity. To obtain a porous scaffold, sol-gel synthesis needs to be associated with freeze-drying, foaming, porogen, 3D sacrificial templates, electrospinning, 3D printing and others.^[38,177-179] These methods allow the control of pore and interconnection sizes with a variable reproducibility depending on the method. No crystallization is observed with such glasses and the composition remains mastered (binary: SiO₂, CaO; ternary: SiO₂, CaO, P₂O₅; etc.). In addition, according to the process at gentle temperature, sol-gel route allows the addition of an organic part to form a hybrid that combines the bioactivity of the BAG and the plasticity of the organic compound. These hybrids are relevant candidates to produce bioactive 3D scaffolds that overcome the high brittleness and the poor resistance to cyclic stress of regular bioactive glasses. Furthermore, sol-gel glass process induces an inherent nano-topography whereas melt-derived glasses are dense. This nano-porosity is

highly favorable for bone cell attachment, proliferation and differentiation which is a critical point for bone regeneration.^[180]

5. Optimization of bioactive glass hybrids

Improvement of the biological activity of biomaterials is a critical aspect in biomaterial engineering. Rather than working around the ratio of different components, more potent investigations may be performed to optimize bone BAG materials through the addition of a doping agent in order to enhance mechanical properties, bioactivity and biological outcomes such as angiogenesis, anti-bacterial properties, bone cell migration, proliferation and differentiation or to limit the impact of the biomaterial on inflammation. Most of the studies have been conducted on melt-derived and heated sol-gel BAG. Accordingly, this section will summarize previous achievement and how inorganic doping could be adapted and even enhanced in association with organic doping in a hybrid system.

5.1. Inorganic doping

Independently of Ca^{2+} and Na^+ ions, it is possible to incorporate other elements (Ag^+ , Sr^{2+} , Cu^{2+} , Zn^{2+} , Mg^{2+} , etc.) into the sol-gel BAG in order to improve their biological properties. (**Table 2**)

However, to our knowledge, no clinical trials have been conducted on inorganic doped-BAG.

Strontium has been extensively studied under the form of strontium ranelate for its effects on bone remodeling cells and was used to treat osteoporosis. It has been shown that this compound strongly improves the activity of osteoblast while repressing osteoclasts.^[181] When incorporated in sol-gel BAG particles (B75-Sr5: 5 wt.% strontium oxide) and tested *in vitro* on murine osteoblasts from calvaria, B75-Sr5 increased the expression of several osteoblastic differentiation markers (Runx2, Osx, Collagen I, etc.) and caused an up-regulation of the alkaline phosphatase enzymatic activity without any cytotoxicity.^[182] Used in the composition of a hybrid monolithic bioactive glass material based on a polydimethylsiloxane(PDMS)- SiO_2 system containing calcium and titanium

(PDMS-SiO₂-TiO₂-CaO-SrO)^[183], strontium increased alkaline phosphatase enzymatic activity in MG63 after 5 days of culture. Unfortunately, this improvement was no longer observable for longer periods, probably due to the decrease in the release of strontium after 7 days of culture.^[184] More recently, melt-derived porous 58S BAG scaffolds ($\pm 55\%$ Si) doped with 1% Sr, 0.3% Li or a combination of both have been implanted in rabbit femoral bone defects. The doped scaffolds showed a better early osseointegration (radiologic and histologic analysis) with the best results for those doped with the two components.^[185] 58S BAG doped with Sr or Li have also shown a positive effect on MC3T3-E1 proliferation and ALP activity with the best results for a proportion of 5 mol% of Li. Moreover, these biomaterials have exhibited antibacterial properties against methicillin resistant *Staphylococcus aureus*.^[186] On the other hand, dissolution products from 45S5 BAG doped with 5% Li have exhibited a pro-angiogenic activity on HUVECs involving the canonical Wnt/ β -catenin pathway.^[187] However, bioactivity is slightly impacted probably due to the substitution of either Li or Sr for Ca.^[186]

Zn-doped sol-gel bioactive glass embedded in a poly-DL-lactic acid (PDLLA) matrix under the form of porous composite scaffolds has also been shown to stimulate ALP activity.^[188] To our knowledge, no biological study has been conducted with Mg-doped bioactive glass materials. These data support both the feasibility of inorganic doping for sol-gel bioactive glasses and their biological relevance for promoting bone cell activities.

For cells to survive and achieve their own functions, the microenvironment of cells must contain enough oxygen and nutrients, while carbon dioxide and cellular waste products must be eliminated. Usually, a higher number of cells are found at the periphery of the scaffolds^[189] and an heterogeneous proliferation is observed due to an oxygen gradient within engineered scaffolds.^[190] Therefore, vascularization and neo-angiogenesis is crucial in bone scaffold engineering and blood capillaries must develop in the scaffolds.^[191] A comprehensive review on this subject has been proposed by Auger et al.^[192] Interestingly, Cu-doped materials present an induction of the production of VEGF.^[193] However a previous study from the same group showed that Cu and Zn

incorporation decreases sol-gel bioactive glass cytocompatibility with human osteoblast-like cells from human osteosarcoma (SaOS-2) depending on the concentration of the BAG dopant.^[188]

According to its ability to stabilize hypoxia-inducible factor 1 α (HIF-1 α) and mimic hypoxic environment^[194] triggering the formation of blood vessels, cobalt-doping is able to induce vascularization in melt derived BAG.^[195] A study from Azevedo et al. shows this stabilization of HIF-1 α protein in human mesenchymal stem cells (MSC) as well as an upregulation of numerous genes involved in hypoxic response (HIF-1 α , HIF2A, VHL), MSC survival (SAG, BCL2), extracellular matrix remodeling (MMP1) and angiogenesis (VEGF, PDGF) in presence of dissolution products from Co-doped melt-derived bioactive glass.^[196] Consistently, Human umbilical vein endothelial cells (HUVECs) have been tested *in vitro* inside hypoxia-mimicking bioactive glass/collagen glycosaminoglycan composite scaffolds. Results showed an increase in the expression and amount of VEGF in cells and these data were corroborated by the enhancement of microtubules formation with Co-doped scaffolds.^[197] To our knowledge, cobalt doping has not yet been achieved with sol-gel bioactive glasses but these data reveal promising results for future studies.

Bacterial infection is the leading cause of surgical reoperation. To address this issue, prophylaxis is the only weapon today and antibiotics are systematically administered to the patient after a biomaterial implantation. Prophylaxis decreases the risk of infections by 80% for total joint arthroplasty.^[198] To further reduce the risk of infection and to avoid a massive use of antibiotics, antibacterial or bacteriostatic biomaterials could be considered. Sol-gel glass particles presenting nano-topography (Mesoporous) and doped with silver ions (SiO₂-CaO-P₂O₅-Ag₂O) have been produced by sol-gel process. The 3-wt% Ag₂O material exhibits bacteriostatic properties against *E. coli* MG1655 with a bacterial growth inferior to 0.01% in the presence of 0.2 mg (biomaterial) per mL (culture solution). The incorporation of Ag₂O did not compromise its bioactivity regarding to bone formation.^[91] A composite scaffold combining poly-DL-lactic acid

(PDLLA) and copper / zinc doped sol-gel bioactive glass particles provides the bioactive glass with additional antibacterial properties against the methicillin resistant, Gram positive, bacillus *S. aureus* which is one of the most prevalent organism in prosthetic-related infections.^[193,198] Unfortunately, Zn^{2+} and Cu^{2+} may act as network modifier ions and decrease apatite-forming ability by increasing the network connectivity. Furthermore, such composites can present non-congruent degradation rates and in that case macro-particle liberation from the structure could raise issues regarding scaffold stability once implanted. A Ga^{3+} -doped sol-gel mesoporous glass synthesized by evaporation-induced self-assembly process (EISA) was proved to induce an apatite-like layer formation after only 1 day in SBF.^[199] The amount of Gallium found in the medium is non-toxic and was effective against *P. aeruginosa*. The effects of Ga^{3+} on the bacterial metabolism could be explained by the fact that Ga^{3+} is replacing Fe^{3+} at important metal binding sites. A recent study showed the *in vitro* osteoinductive potential of gallium-doped mesoporous bioactive glass on mouse pre-osteoblastic cell line (MC3T3-E1). In this work, authors highlighted a positive effect of the incorporation of gallium on pre-osteoblasts differentiation. On the other hand this gallium-doped material showed a repressive effect on the differentiation of the osteoclastic cell line (RAW 264.7).^[200] A particular attention must be given to the repression of osteoclastogenesis since their activity is crucial for the durability of a biomaterial. The right balance between bone formation and resorption is essential for a complete healing.^[201] This biological balance can be evaluate *in vivo* by an histologic evaluation.^[202] However, most of these studies have been directed on melt-derived or sol-gel BAG involving heat treatments that are not compatible with the addition of many synthetic polymers and most natural polymers during the sol-gel process. Fortunately, inorganic doping can be transposed to room temperature organic-inorganic hybrids upon synthesis adaptations, allowing the combination with an organic doping, including compounds sensitive to heating.

5.2. Organic doping and beyond

Bone remodeling is affected by many biological factors such as parathyroid hormone^[203], calcitriol^[204], thyroid hormones (calcitonin, etc.)^[205], growth hormone^[206], glucocorticoids^[207] and sex hormones^[208–211], insulin-like growth factors (IGFs)^[212], tumor growth factor-beta (TGF-beta)^[213], bone morphogenetic proteins (BMP)^[214], prostaglandins and cytokines,^[215–217] etc. Many cytokines and growth factors participating in bone remodeling either locally or at the body scale can be considered as doping agents. **(Table 3)** BMPs are the most studied for biomaterial doping.

Most of the sol-gel BAG require heating to incorporate calcium and phosphate in the silicate network. Thus, so far, most of the organic doping have been achieved by adsorption at the surface of the biomaterials rather than being integrated within the core of the biomaterial. Adsorption of doping molecules relies on the surface topography and chemistry of the material. Sol-gel BAG has shown an inherent mesoporosity that is a crucial parameter for the adsorption and release of doping molecules. To date, mesoporous bioactive glass scaffolds have significantly higher loading efficiency and more sustained release of VEGF than non-mesoporous ones. This method of VEGF release preserves the biological activity of the protein as demonstrated by an improved viability of endothelial cells cultivated in a VEGF-loaded mesoporous BAG.^[218] Accordingly, Tang's group tailored a 7.5 nm, 3D cubic (Im3m) mesoporous structure for a "size-matched entrapment" of rhBMP-2 to achieve a sustained release and a preserved bioactivity. In their hands, rhBMP-2-loaded BAG induced an excellent cell attachment, ingrowth and osteogenesis *in vitro* and allowed a complete bone reconstruction *in vivo* in a rabbit radius critical size defect model.^[219] The same trends has been demonstrated with magnesium-zinc-silicon gels having a pore size of ~4 nm. They exhibit a sustained release of rhBMP-2 promoting MG63 cell proliferation and differentiation as compared with a control biomaterial without mesopores.^[220]

These overlapping adsorption strategies greatly support the interest in doping bone substitutes with organic compounds for clinical application including the development of future orthopedic and dental biomaterials. Interestingly, combining mesoporosity with a 3D structure leads to a greater contact surface, thus increasing loading and release capacity of the biomaterial.

However, adsorption leads to uncontrolled release and may enhance inappropriate biological behavior. Actually, adsorption rapidly creates a burst of release after implantation.^[220] Studying the influence of a mesoporous bioactive glass scaffold loaded with dexamethasone on proliferation, differentiation and gene expression of human osteoblasts, Wu et al. reported that about 50% of the dexamethasone were released within 24 hours, thus enlightening the dissociation between both the biomaterial degradation and the drug delivery rates.^[221] In a Korean study from Lim et al., 3D BAG scaffolds obtained with sacrificial microfibers templates were obtained and loaded with dexamethasone. These scaffolds enhanced the differentiation of human dental pulp cells and several metabolic pathways were proposed to be responsible of this effect (Integrins, mTOR: mammalian target of rapamycin and BMPs).^[222] Recently, IL-8 and BMP-2 were orchestrated in a mesoporous bioactive glass-based spatiotemporal delivery system to achieve a rapid release of IL-8 followed by a long-term sustained release of BMP-2. Briefly, BMP-2 was adsorbed at the surface of the BAG then coated with PEG. IL-8 was then adsorbed on the coating. With this spatiotemporal delivery system, IL-8 and BMP-2 induced a rapid and efficient stem cell recruitment and a “chondrogenic/osteogenic balance” at the first stage of endochondral ossification. The scaffold exhibited sufficient osteoconductivity, resulting in early extensive bone regeneration both *in vitro* and *in vivo*.^[223] Although smart, this system does not fully address the phasing of the degradation rate of the different components.

The sol-gel synthesis pathway^[224] has dramatically increased the potential of BAG but calcium incorporation remained an issue until recently. New advances in sol-gel chemistry (see: sol-gel hybrid section) now permit to process sol-gel bioactive glass scaffolds with a homogeneous incorporation of calcium completely at room temperature. These improvements allow considering the incorporation of organic dopants homogeneously inside the BAG network and offer new avenues in the design of organic doped BAG hybrids. Such “in mass” strategies support a sustained release of doping molecules and address the phasing of the degradation rate of the different

components. In this case, the biomaterial releases the doping agent as it degrades consistently with the formation rate of new bone tissue.

However, according to the controversy reported on the use of growth factors such as BMPs for biomaterial doping, alternative osteoinductive factors without side effects must be investigated to warrant the safety of these innovative strategies. ^[225–228] Accordingly, our group has just started a research program on the potential of nutrients (known for their ability to promote osteoblastogenesis and subsequent bone formation) as doping molecules for biomaterials. Experimental procedures are ongoing but such approaches may represent an innovative and timely alternatives in the “quest for the bone graft”.

Section on animals models that would help deciphering the biological relevance of the tested biomaterials?

6. Conclusion

Despite a wide range of data and a great number of innovative projects (around twenty ongoing clinical trials based on BAG implantation) , scientists and physicians are still looking for the Holy Grail in the field of biomaterials for bone regeneration. While most biomaterials are promising *in vitro*, pre-clinic and clinic evaluations remain too poorly reported and surgeons keep on using regular unsatisfying biomaterials. In this light, sol-gel bioactive glasses and their subsequent organic-inorganic doped hybrids may present a particular interest but their clinical relevance now needs to be assessed.

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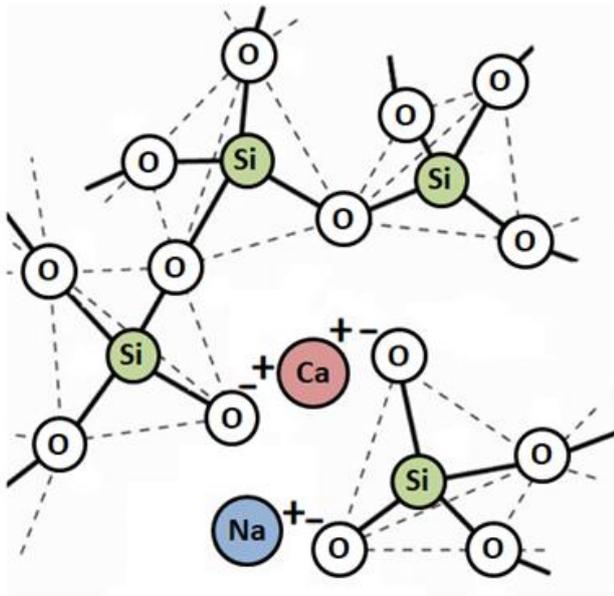


Figure 1. Silicate network with network modifiers

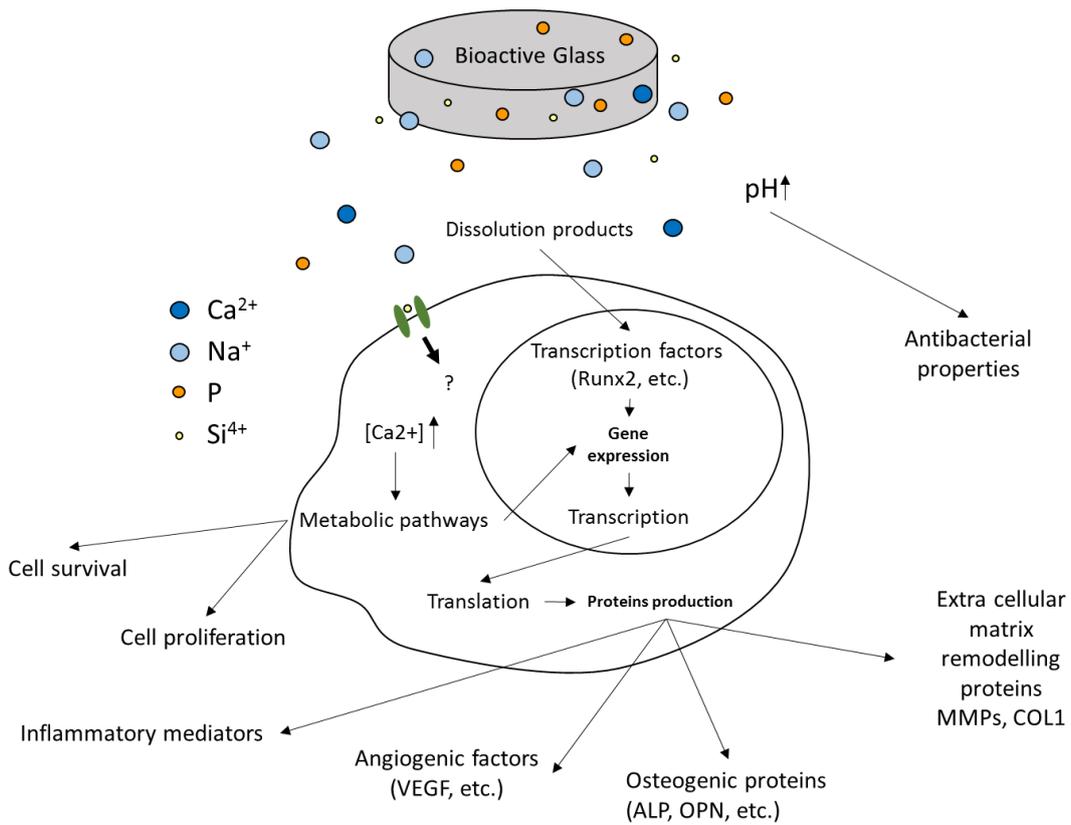


Figure 2. Biological properties of a bioactive glass

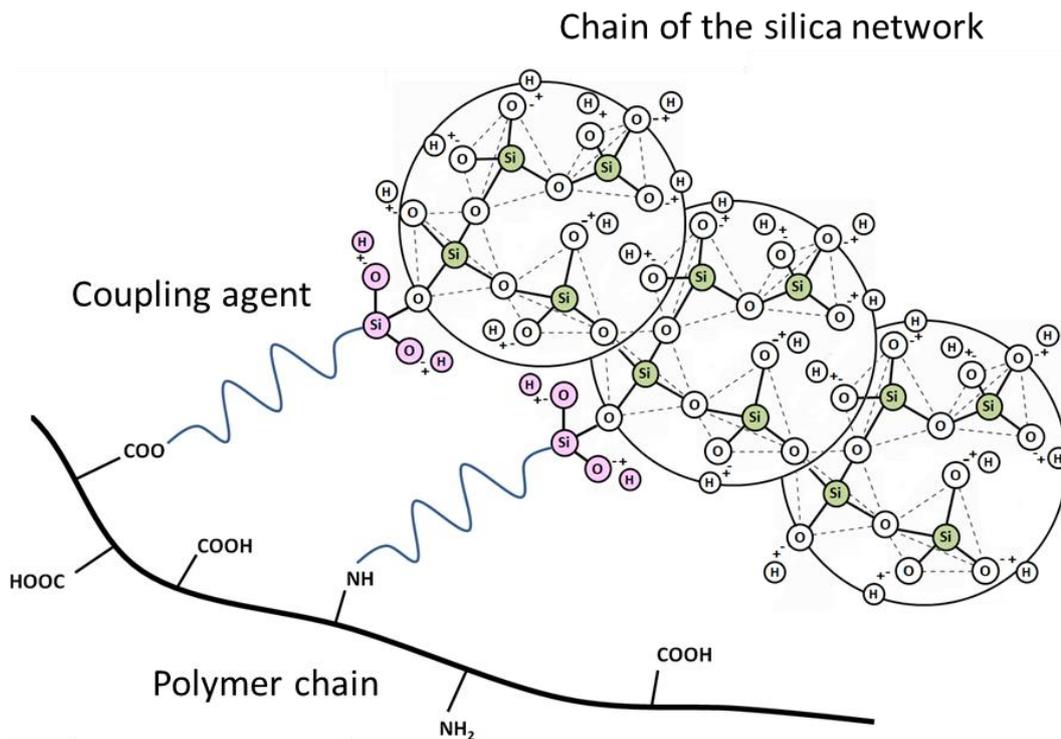


Figure 3. Cross-linking between organic and inorganic phase leading to a class II hybrid



Figure 4. Gelatin/BAG hybrid scaffolds, a) under different shapes; b) observed in scanning electron microscopy.^[164]

Table 1. Comparison between composite and hybrid materials

	Composites	Hybrids
Phase relationship	Different phases	Interpenetrated network
Phase dispersion	Non homogenous	Homogenous
Apatite nucleation	Non homogenous	Homogenous
Dissolution behaviour	Non homogenous (instability)	Homogenous (stability)

Table 2. Non exhaustive list of inorganic compound used for BAG doping

Doping agent	Biologic effects	References
Sr	Improve osteoblasts functions Repress osteoclasts activity Improve early osseointegration Antibacterial properties	[182–186]
Mg	Improve bioactivity Improve mineralization	[229,230]
Cu	Promote angiogenesis Antibacterial properties	[188,193,231,232]
Co	Promote angiogenesis	[195–197,233]
Zn	Stimulate ALP activity Antibacterial properties	[188,193]
Ag	Antibacterial properties	[91]
Ga	Improve bioactivity Antibacterial properties Improve osteoblasts functions Repress osteoclasts activity	[199,200]
Li	Stimulate ALP activity Promote angiogenesis Improve early osseointegration Antibacterial properties	[185–187]

Table 3. Non exhaustive list of organic compound used for BAG doping

Doping agents	Biologic effects	References
VEGF	Promote angiogenesis	[218]
BMP-2	Improve osteoblasts functions	[219,220,223,234,235]
Dexamethasone	Improve osteoblasts functions	[221,222,236–239]
IL-8	Improve osteochondral ossification	[223]
Gentamicin	Antibacterial properties	[240,241]
Dimethylallyl Glycine	Promote angiogenesis	[235,242]



Henri GRANEL performed a thesis in the field of bone regenerative medicine. During this period, he worked on bioactive glass based hybrid scaffolds for bone defects repair. Now, he is employed as a post-doctoral fellow and still works on these hybrid biomaterials but also on calcium phosphates.

Despite a wide range of commercially available biomaterials for bone tissue repair, they mostly remain not completely satisfying. Bioactive glasses (BAG) are among the most promising biomaterials for bone defects repair. This review focuses on BAG evolution from the original 45S5 to hybrid scaffolds combining BAG and polymers. This review timely considers recent strategy designs presenting their advantage and limitations and offer new insight in the field that support innovative opportunities.

Keyword: Bone regeneration

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