Drug delivery using composite scaffolds in the context of bone tissue engineering

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Summary

Introduction. **Due to the disadvantages of the current bone autograft and allograft in many clinical condition in which bone regeneration is required in large quantity, engineered biomaterials combined with growth factors, such as bone morphogenetic protein-2 (BMP-2), have been demonstrated to be an effective approach in bone tissue engineering, since they can act both as a scaffold and as a drug delivery system to promote bone repair and regeneration.**
Area covered. **Recent advantages in the field of engineered scaffolds have been obtained from the investigation of composite scaffolds designed by the combination of bioceramics, especially hydroxyapatite (HA), and biodegradable polymers, such as poly (D,L-lactide-co-glycolide) (PLGA) and chitosan, in order to realize osteoconductive structures that can mimic the natural properties of bone tissue. Herein it is demonstrated that the incorporation of BMP-2 into different composite scaffolds, by encapsulation, absorption or entrapment, could be advantageous in terms of osteoinduction for new bone tissue engineered scaffolds as drug delivery systems and some of them should be further analyzed to optimized the drug release for future therapeutic applications.**
Expert opinion. **New design concepts and fabrication techniques represent novel challenges for further investigations about the development of scaffolds as a drug delivery system for bone tissue regeneration.**

KEY WORDS: bone morphogenetic protein-2; bone tissue engineering; composite scaffold; drug delivery.

Introduction

Bone regeneration is a complex, well-orchestrated physiological process, involving a number of cell types and intracellular and extracellular molecular signalling pathways which can be seen during normal fracture healing and are involved in continuous remodelling throughout adult life, in an effort to optimise skeletal repair and restore skeletal function. There are numerous clinical conditions in which bone regeneration is required in large quantity, such as skeletal reconstruction of large bone defects created by trauma, infection, tumour resection and skeletal abnormalities, or cases in which the regenerative process is compromised, including avascular necrosis, atrophic non-union and osteoporosis. Moreover, with the increased life expectancy and the consequent aging of population, a stimulation of bone healing to reduce and treat complications seems to be necessary.
Currently, the gold standard of clinical therapeutic strategies to enhance bone regeneration is the use of autologous grafts, which represent the "ideal bone graft substitutes", since they combine all necessary features to induce bone growth and regeneration: osteogenic cells, osteoinductive and osteoconductive properties. Furthermore, because it is the patient's own tissue, autologous bone is histocompatible and non-immunogenic, reducing to a minimum the likehood of immunoreactions and transmission of infection (1). Although these properties, autogratfs are limited and donor site morbidity, caused by additional surgical procedures for harvesting, accompanies by the risk of infections, haematoma and chronic pain, is common and well-documented since all of these complications may lead to implant failure (2-6). An alternative approach is represented by allogenic bone grafting, obtained from human cadavers or living donors, which bypasses the problems associated with harvesting and quantity of graft materials but still has other limitations like transmission of diseases from donor to recipient or immunogenic reactions (7, 8). As a result of the limited successes of auto- and allografts in some clinical situations, including non-union defects in long bones and bone loss following trauma or tumour resection, tissue engineered bone substitutes have been investigated extensively as a promising therapy.
Bone tissue engineering is a recent field of research associated with regenerative medicine and has been defined as "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function" (9). At present, most studies are focused in the development of porous 3D structures, named scaffolds, following the concept of biomimicry (literally defined as the imitation of life or nature) to more closely mimic the anatomical and biochemical organization of cells and matrix native to achieve the suitable mechanical properties for the tissue (10). In fact, it is important for tissue engineers to understand the biological events and signals involved, for instance, in musculoskeletal cell and tissue morphogenesis

since it may be necessary to employ the principles of developmental biology in designing the appropriate microenvironment for tissue regeneration.
There are multiple physical and biological requirements that an ideal bone scaffold should address:

1) provide temporary mechanical support to the affected area;
2) act as a substrate for osteoid deposition;
3) contain a porous architecture to allow for vascularization and bone in-growth;
4) encourage bone cell migration into the scaffold;
5) support and promote osteogenic differentiation in the non-osseous, synthetic scaffold (osteoinduction);
6) enhance cellular activity toward scaffold-host tissue integration (osseointegration);
7) degrade in a controlled manner to facilitate load transfer to developing bone;
8) produce non-toxic degradation products;
9) not incite an active chronic inflammatory response;
10) be capable of sterilization without loss of bioactivity;
11) deliver bioactive molecules, such as growth factors or drugs, in a controlled manner to accelerate healing and prevent pathology (11).

Numerous porous materials have been investigated, but despite substantial progress in the field, the realization of synthetic structures, able to fully harness the bone's capability to regenerate and remodel itself and mimic the complicated physiochemical attributes of bone, still presents challenges. In particular, much interest has been addressed in enhancing the functionality of the scaffolds, by loading biomolecules, such as growth factors or drugs, into them to treat bone disorders or to act on the surrounding tissues with an adequate therapeutic concentration level and for a desired time frame, because it is recognized as being highly beneficial, hence the increasing interest in incorporating a drug-delivery function in tissue engineering applications (12).
Three-dimensional bone bioactive scaffolds can be fabricated from a wide variety of bulk biomaterial, but especially bioceramics [HA, tricalcium-phosphate (TCP), bio-glasses (BG)] and biodegradable polymers, natural or synthetic [collagen, fibrin, chitosan or polyesters, polyethylene glycol (PEG), polydioxanone] are considered to be potential scaffolds. In particular, their composites represent an optimized and convenient alternative as they combine the advantages of both bioactive ceramics and biodegradable polymers for bone tissue engineering. In fact, ceramics fail mechanically due to brittleness (hard material with small elongation to failure), whereas in polymers there is a deficiency in the compressive modulus compared with native bone tissue (polymers typically too soft) (13). This discrepancy may be reduced or eliminated through producing composite formulations designed to combine the advantageous properties of multiple materials.
As previously introduced, major advancements in bone tissue engineering are achieved through integrating biomolecules which made the scaffold more osteoinductive. Biomolecules integrated into the scaffold could be represented by proteins/growth factors, such as transforming growth factors-βs (TGF-βs), bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs), platelet-derived growth factors (PDGFs), vascular endothelian growth factor (VEGF), since these factors control osteogenesis, bone tissue regeneration and extracellular matrix via recruiting and differentiating osteoprogenitor cells to specific lineages (14). Therefore, incorporating different growth factors and other biomolecules are of special interest for bone tissue engineering.
In this review, we will give an overview about different kinds of composite scaffolds used in bone tissue regeneration, that can be work not only as a temporary mechanical support to the affected area, but also as a drug delivery, in order to promote and facilitate bone regeneration.
We focus on recent advantages in biomolecule-incorporated composite scaffold and their osteogenic properties. We first discuss on growth factors that can be loaded in composite scaffold to help bone healing, in particular on BMP-2. Then we discuss about composite scaffold developed as drug delivery to optimize the release of the bioactive molecules. Finally, we conclude with critical issues and future developments of scaffolds for next generation bone tissue engineering.

Growth factors for bone tissue engineering

One possibility to improve the osteoinductivity of a scaffold used for bone tissue engineering is the application of biologically active molecules. A common strategy is to utilize growth factors that act on a wide variety of cells and direct their actions via cell-surface receptor binding and activation (15). They naturally occur within a health bone matrix or are expressed during fracture healing to direct the development of structures, vascularization and differentiation of bone cells; such growth factors are for example TGF-β, BMPs, IGFs, PDGF, VEGF (16-19). In animal models, it has been shown that introducing specific biomolecules can enhance the union of non-union type (a fracture that does not heal by itself after several months) bone fractures (20).
In particular, of these molecules, BMPs, members of the TGF-β superfamily, have been the most extensive studied, as they are potent osteoinductive factors: they induce the mitogenesis of mesenchymal stem cells (MSCs) and other osteoprogenitors, and their differentiation towards osteoblasts (21, 22). There is a number of experimental and clinical trials supporting their safety and efficacy and, for that reason, they represent a very promising candidates for the treatment of bone diseases and defects (23-25). More than 15 BMPs have been identified in vertebrates to date and, among these, BMP-2 has been shown to be one of the most potent inducers of bone formation *in vivo* (26-28). Moreover, with the use of recombinant DNA technology, BMP-2 has been licensed for clinical use since 2002 (19) and has been used in a variety of clinical condition including non-union, open fractures, joint fusions, aseptic bone necrosis and critical bone defects (29).
Current clinical strategies involve the combination of recombinant human BMP-2 (rhBMP-2) with an absorbable collagen sponge (30-33) and make the growth factor delivery a promise in clinical bone repair. Extensive research is focusing to develop new formulation for minimally invasive application and/or novel carrier for prolonged and targeted local

delivery (34) and the clinical use of BMP-2, either alone or combined with bone grafts, is in constantly increasing. However, there are several issues about its use: the existing collagen-based BPM carriers use highly supraphysiological doses (on the order of milligrams) needed to obtain the desired osteoinductive effects, considering also the short i*n vivo* half-life of growth factors, delivered over relatively short timescale, which has led to well-documented clinical side effects including edema, ectopic bone formation, potentially increased cancer risk and the high cost of treatment (13, 35-38).

Since BMPs are labile and expensive proteins, some research groups have reported studies about short peptide sequences of the core regions that could mimic BMPs in terms of bone regeneration, suggesting that they can be used as an alternative inducer. In some studies, they synthetized a 24-amino acid peptide derived from the "knuckle epitope" of BMP-2 and found that the peptide can precisely regulate biological behaviours of cells, such as adhesion and differentiation. Meanwhile, it also owns excellent osteoinductivity and ectopic bone formation property *in vivo*, which is similar to those of BMP-2 (39, 40).

Emerging approaches have focused on controlling growth factor release kinetics in order to decrease the needed dose and limit deleterious side effects. Sponges (24), hydrogels (41, 42), particulates and various micro/nano carriers (42) have been used to successfully deliver bone growth factors over longer timescales than the collagen sponges used in current clinical applications. However, these approaches tend to use carrier materials that are not structurally optimized for bone tissue engineering applications and some strategies may be difficult to translate to clinical applications. Therefore, there is a need for clinically relevant strategies that can deliver bone growth factors over sustained, controllable timeframes from scaffold with optimized structural properties.

The main role of a delivery system for BMP-2 is to detain the growth factor at the site injury for a prolonged time frame, providing an initial support for cells on which they can attach and form regenerated tissue (43). The scaffold should be biodegradable to allow the formation of an interface with surrounding biological tissue and for complete invasion of the healed tissue, and presents adequate porosity to permit the infiltration of cells and formation of blood vessels at the new bone; for that purpose the scaffold should provoke an optimal inflammatory response. Furthermore, the scaffold should act as a carrier to protect the BMP-2 from degradation and has to maintain its bioactivity while realising the cytochine in a time – and space – controlled manner to promote the regeneration of new bone at the implant site.

In general, the immobilization of growth factors, such as BMPs, in a delivery system may be performed by different methodologies: via adsorption, entrapment or immobilization, or by covalent binding (44, 45). In case of adsorption, impregnation of delivery matrix with the growth factor is simpler but conformational changes might occur and the release of the protein be less sustained. Furthermore, delivery by adsorption often results in initial burst release (46). With entrapment methodology into a polymeric matrix, the growth factor is immobilize and release over extended periods of time. However, there is a lot of difficulties associated to this, because during the process can occur variations of pH and temperature that can led to the denaturation of the protein and, consequently, to the loss of their activity (47). Lastly, the BMPs may become immobilized by covalent binding to the carrier; this is performed by producing a fusion BMP protein with a domain of specific binding to a biomaterial (47).

Another novel strategy to prolonging viable growth factor release from polymers, especially from PLGA, is to conjugate the copolymer with heparin, a highly sulphated glycosaminoglycan. Since heparin is known to bind growth factors, several groups have incorporate it into PLGA scaffolds to immobilize growth factors and prevent diffusion thereby prolonging their viable release (48, 49). This strategy has proven to be very successful and holds promise for the future of growth factor delivery systems for bone regeneration.

Taking in mind all of these factors, in the next paragraph, we are going to analyse particular composite scaffold, from recent literature, that can be taken in consideration as a drug delivery system for BMP-2 and be employed in bone tissue engineering for future clinical applications.

Composite scaffolds as a drug delivery in bone regeneration

The continuing research for new bone scaffold materials is driven by the need to exceed the shortcomings of existing materials, ceramics and polymers, that may show limited mechanical properties required for temporary bone substitutes. These issues may be reduced or eliminated through the mixing of polymers, natural or synthetic, and inorganic components, like HA, TCP and BG; it represents a convenient approach to prepare an alternative composite scaffolds that can successfully be used in bone tissue engineering since they combine the advantages of both biodegradable polymers and bioactive ceramics (13). In particular, bioactive inorganic materials, such as HA, BG, TCP, induce the effective interaction of the scaffold with the surrounding bone tissue by forming a tenacious bond via the growth of a carbonate HA layer on its surface that significantly enhances osteoblast activity and adsorbs proteins and growth factors that facilitate new bone formation (50).

Moreover, addition of inorganic materials to bioresorbable polymers can change the polymer degradation behaviour by buffering the pH of the nearby solution, preventing the autocatalytic effect of the acidic end groups resulting from hydrolysis of polymer chain (for example in polylactic acid) and limiting the local acidic environment that can also have adverse tissue responses (51).

In many composite scaffolds, the matrix is usually prepared by using biodegradable polymers and inclusions, in the form of particles or fibres, of HA, TCP or BG to improve the mechanical strength and bioactivity (52-55). Furthermore, over the past years, many release dosage forms have been developed for drug or protein delivery, like nanoparticle and microspheres. However, one common problem is the existence of a large burst over a narrow time period during the early stage of release. As a strategy to solve that issue, Nie et al. (56) have taken in consideration in their study fibrous PLGA)/nanoparticles HA (nHA) composite scaffold as a better release dosage form because of its favourable properties and morphology (57). Compacted fibrous scaffold, compared with microsphere, can give cell stable three-dimensional growth environment an provide a good support to the new generated bone. Thanks to the fabrication technique, electrospinning (58), Nie et al. encapsulated into fibrous PLGA/nHA scaffold the rhBMP-2, investigating the effect of HA content on the biological and physical characteristics of

the scaffold fabricated. They showed that rhBMP-2 encapsulated into fibres retained its integrity and structural conformation *in vitro*. Moreover, the use of HA as additive can aid the release of rhBMP-2 from fibres while it also protects the growth factor from denaturation through the contact with organic solvent used in electrospinning technique; the nHA, thanks to its hydrophilicity, makes BMP-2 to elude the contact with the organic solvent during the fabrication process and preserve its integrity. Following studies on cell attachment and cytotoxicity showed that the incorporation of nHA in the fibrous scaffold can enhance cell attachment and viability, making the composite scaffold a good model to be better studied *in vivo* for BMP-2 delivery and bone regeneration.
It is interesting to note that for promoting the ability of cell adhesion to the nHA/PLGA composite scaffold, Zhang et al. (59) have investigated a new strategy for the use of the tripeptide arginine-glycine-aspartic acid (RGD) on biomaterials, since it is an amino acid sequence that actively promotes cellular adhesion through binding to integrin receptor (60, 61), developing a novel scaffold with the incorporation of RGD peptides, in the form of conjugated copolymer, and BMP-2 into the porous composite scaffold for promoting cell adhesion, growth, differentiation and thus bone regeneration.
Given that HA and collagen are the main components of natural bone, the HA/collagen composite scaffold material is being extensively studied (62). Moreover, since poly(L-lactic acid) (PLLA) is a non-toxic and degradable biomaterial widely used as a scaffold material in bone tissue engineering, a novel nHA/collagen/PLLA bone tissue engineering composite has been successfully fabricated (63-67). This new scaffold improves cell attachment and stimulates cell proliferation and differentiation due to its main composition and hierarchical microstructure closely resembling those of natural bone (65). In particular, Li et al. (20) have prepared a nHA/collagen/PLLA composite loaded with the rhBMP-2 and with a novel short BMP-2-related peptide that could regulate adhesion and differentiation of bone marrow stromal cells and induce ectopic osteogenesis (68, 69), with the aim to overcome the BMP-2 drawbacks, such as instability and costs of high-doses therapies. They analysed the *in vitro* release of BMP-2-related peptide: after the initial burst, the peptide was release in a sustained and controlled manner for 12 weeks, following the slow degradation of the scaffold. Then, they have evaluated whether this scaffold may be used in the surgical treatment of bone defects, making studies and observing new bone formation *in vivo*. They created a rat circular cranial bone defect (5 mm in diameter) using a trephine drill and implanted the scaffolds in the full-thickness bone defect. They sacrificed the rats at 6 and 12 weeks after the surgery for radiographic evaluation and histological examination. They found that both of the two scaffold BMP-2 and, in particular, BMP-2-related peptide improved the osteoinductivity of nHA/collagen/PLLA and efficiently promoted bone defect repair with respect to the scaffold without the growth factors, suggesting it not only as a promising scaffold material for bone tissue regeneration and drug delivery but also confirming the BMP-2 related peptide as a good substitute of the respective and more expensive BMP-2.
Some research groups have developed a microspheres-scaffold system with the capacity of releasing bioactive peptide in a well-controlled manner using chitosan microspheres. This hydrophilic polysaccharide has been widely used for the controlled delivery of polypeptides and proteins in the format of microspheres or nanoparticles (70-72); in fact the release kinetics can be modulated by adjusting the factor loading amount, chitosan molecular weight and preparation methods. Hou et al. (73) have recently studied a new composite scaffold combined adsorbable collagen sponge to chitosan microspheres adsorbed with rhBMP-2. Adsorbable collagen sponge approved by the FDA was used to load rhBMP-2 but the initial burst release of protein (within the first days 80.32%) was not beneficial for bone healing. For that reason, Hou et al. investigated a superior carrier material for sustained release. The chitosan microspheres they have used clearly showed a polyporous morphological structure that can adsorb more protein than those without a polyporous structure. Previous studies have demonstrated that carriers equipped with adequate porosity allow higher adsorbability (47). The adsorbable collagen sponge/chitosan scaffold loaded with rhBMP-2 has exhibited minimal initial burst release (47.63%) followed by moderate release, making that scaffold as an optimal and ideal carrier of rhBMP-2. Moreover, *in vivo* studies performed on white rabbits after implantation of the scaffold in the middle of the defective radius, indicated that the implant possesses osteoinductive properties, the rhBMP-2 maintained its bioactivity and a better regeneration efficacy of the bone defects after 12 weeks post implantation, respect to the adsorbable collagen sponge with or without rhBMP-2.
Another research group, Niu et al. (74) have utilized in their work a synthetic peptide derived from BMP-2 designed by Duan (40) and encapsulated it into the chitosan microspheres preparation. Afterwards, the microspheres were blended in a solution of nHA/collagen/PLLA for fabricating a three dimensional porous scaffold, able to act as a delivery carrier too. The bioactivity of the encapsulated BMP-2 derived synthetic peptide was totally preserved during the process of scaffold preparation, as reported in the *in vitro* studies, monitoring the expression of alkaline phosphatase activity of mesenchymal stem cells cultured with the condition medium containing the released synthetic peptide. Moreover, the synthetic peptide release kinetics from nHA/collagen/PLLA/chitosan microspheres was governed by degradation of both incorporated chitosan microspheres and PLLA matrix until 20 weeks, making that scaffold a good model to be used to deliver bioactive factors for a variety of tissue regeneration applications.
Polymers combined with ceramics particles, such as HA, can also be applied as coating on porous bioceramic scaffolds, in order to tailor the controlled release of a drug (75). A recent study by Jun et al. (76) shows a silica containing 30% chitosan as hybrid coating material to incorporate BMP-2 on a porous HA scaffold, in order to evaluate the release behaviour of the growth factor from the scaffold and its *in vivo* performance for bone tissue engineering. The silica-chitosan hybrid coating was demonstrated as an osteoconductive vehicle that controls the release behaviour of growth factors for a long period of time; moreover this hybrid has many advantages for delivering growth factors, since it has a highly porous structure and biodegradable properties. Also, it is synthetized at room temperature and so the incorporation of growth factors into the hybrid in an *in situ* manner is facilitated because there is not protein denaturation (77, 78). Jun et al. evaluated the release behaviour of the BMP-2 from the coating up to 42 days and they showed no initial burst and a sustained release of the growth factor for 6 weeks. That result can be explained as a consequence of the good affinity of the hybrid for BMP-2, making the hybrid coating as a good carrier material for sustained BMP-2 delivery, effectively re-

ducing the needed amount of BMP-2 in bone regeneration. Moreover, *in vivo* experiments, performed on rabbit calvarial defect model, showed the effective bone forming ability of the BMP-2 loaded hybrid coating HA scaffold in comparison to the pure porous HA scaffold, making it a good candidate for future therapeutic application.

Conclusion

In reviewing the published studies from recent literature on composite bone tissue engineered scaffolds with additional drug delivery capability, it is clear that there have been continued advances towards the further development of the field.

Herein, we have reported works that take in consideration the combination of HA with specific polymers, such as PLLA, PLGA and chitosan, since they represent the much studies and promising materials in the field of bone regeneration thanks to their own physical and mechanical properties.

Different kind of composite scaffold, loading BMP-2 by encapsulation, by adsorption or by entrapment into the fibrous scaffold using electrospinning technique, have been investigated and have shown good results in terms of osteointegration and drug release, but a cause of the limited data on the *in vivo* studies, further efforts and challenges will be necessary to optimize the kinetics release of the growth factor with the aim to fabricate suitable models to be effectively employed in the future clinical and therapeutic applications.

Expert Opinion

New design concepts and fabrications technique are urgently need to develop novel scaffolds for bone tissue regeneration. In fact, the disadvantages of current bone autografts and allografts in many clinical condition in which bone is required in large quantity, limit the successful of their therapeutic applications and may lead to implant failure.

In the last years, the research has been focused on the realization of three dimensional porous scaffolds which can mimic the architecture and possess the mechanical and physical properties of native bone, supporting the numerous cell types that naturally occur during the process of tissue regeneration and development, and the new tissue ingrowth. For that purpose, composite scaffolds represent excellent candidates thanks to the combinations of two or more bulk materials that improve their characteristics in terms of toughness, strength, elasticity, porosity, biocompatibility, controlled biodegradation and especially osteoconductivity.

Several attempts have been made to include growth factors and proteins within the bioactive scaffolds to make it also osteoinductive, working not only as a support but also as a drug delivery system, stimulating cellular adhesion, proliferation and differentiation, in order to promote and facilitate bone regeneration. In addition, enhancing the functionality of these already complex matrices by loading drugs into them to treat bone disorders or to act on the surrounding tissue with an adequate therapeutic concentration level and for a desired time frame, represents a very stimulating and challenging field for bone tissue regeneration.

It is therefore expected that this field will keep growing the next years and it would be important to intensify the working collaboration of researchers and technologists, considering the interdisciplinary character of tissue engineering, to overcome presents limitation of techniques that are used to synthetized the new smart scaffolds.

Despite significant efforts in this direction, several challenges have yet to be resolved. These include understanding the many variable conditions during the scaffold processing and the physic-chemical properties of the novel three dimensional delivery system with special considerations to the stability of the incorporated drug. In particular, many methods of fabrications, such as encapsulation of growth factors in polymeric scaffold, utilize organic solvents that can be residual in the matrix after processing and could limit not only the amount of active loaded drug but also the future cellular adhesion to the scaffold. Moreover, the hot temperature during these processes should be controlled to avoid the denaturation of the bioactive proteins and to preserve their functionality. Further investigations on drug release and its kinetics need to be deepen in order to design a tightly controlled temporal and spatial long-term release profiles.

Many novel composite scaffolds have appeared and they still continue to appear, considering the wide choice of bulk materials with interesting features that could be used in tissue regeneration.

Bioceramics, and especially HA, in combination with polymers have been extensively investigated for applications in bone tissue engineering and, although they represents a very promising material for future clinical applications, in the last five years, the use of mesoporous bioactive glass (MBG) for drug delivery and bone tissue regeneration has grown significantly because of the excellent behavior.

MBG is an inorganic material base on a $CaO\text{-}SiO_2\text{-}P_2O_5$ composition and it has highly ordered mesopore-channel structure. It possesses a more optimal surface area, pore volume, improved *in vitro* apatite mineralization in simulated body fluid and excellent cytocompatibility (79). These features make it have a superior bioactivity as well as one of the best candidate to be used as a platform for drug delivery, thanks also to the existence of a large number of Si-OH groups that can be functionalized (80, 81). Many studies have shown the bioactivity of MBG can be improved incorporating it into polymers such as poly-caprolactone (PCL) (82), PLGA (83) and silk (84), and it is realistic to declare that, within the next few years, MBG will be tested widely in large animal models and possibly used for potential application as a drug delivery system, opening new challenges in bone tissue engineering for future and better investigations.

Article highlights box

- Many shortcomings exist in the traditional methods of treating bone defect, such as donor tissue shortage for autografts and disease transmission for allografts.
- Bone tissue engineering have provided promising ways to repair and replace damaged bone thanks to composite scaffolds that can be consider not only as a temporary support but also as a drug delivery system.
- Bioactive molecules that can be integrated into scaffolds are proteins/growth factors such as TGF-β, BMPs, IGF, VEGF. Among them, BMP-2 represents a very promising candidate since it is a very potent osteoinductive factor.
- Recent research groups have investigated novel scaffolds composed by HA and polymers, such as chitosan, PLGA and PLLA in which BMP-2 is loaded by encapsula-

tion, adsorption or entrapment into polymeric fibers, trying to preserve its bioactivity and supporting the controlled release in order to make easier the process of bone regeneration.

- Although they represent promising scaffolds, further investigations in the field to optimize the growth factor release and current methods of fabrication, with *in vivo* studies, will be necessary and will represent new challenges for future clinical applications.

Declaration of interest

Nothing to declare.

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