REVIEW ARTICLE

Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery)

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Abstract

Bone morphogenetic proteins (BMPs) are cytokines with a strong effect on bone and cartilage growth and with important roles during embryonic patterning and early skeletal formation. BMPs have promising potential for clinical bone and cartilage repair, working as powerful bone-inducing components in diverse tissue-engineering products. Synthetic polymers, natural origin polymers, inorganic materials and composites may be used as carriers for the delivery of BMPs. Carriers range from nanoparticles to complex three-dimensional (3D) scaffolds, membranes for tissue-guided regeneration, biomimetic surfaces and smart thermosensitive hydrogels. Current clinical uses include spinal fusion, healing of long bone defects and craniofacial and periodontal applications, amongst others. BMP-2 and BMP-7 have recently received approval by the US Food and Drug Administration (FDA) for specific clinical cases, delivered in absorbable collagen sponges. Considering the expanding number of publications in the field of BMPs, there are prospects of a brilliant future in the field of regenerative medicine of bone and cartilage with the use of BMPs. Copyright © 2008 John Wiley & Sons, Ltd.

1. Introduction

Every year millions of surgical operations are performed for the healing or repair of an organ. In the past two decades, tissue engineering has emerged as a very promising alternative that circumvents several of the limitations of the existing options of autografting and allografting for the treatment of a malfunction or lost body part. Tissue engineering combines precursor cells from the patient with scaffolding matrices and the stimulus of

growth factors. Since the advent of tissue engineering, bone has received particular interest, since it is one of the tissues with most regenerative abilities in the human body.

Bone morphogenetic proteins (BMPs) are probably the most important growth factors in bone formation and healing (Reddi, 1998, 2005). These cytokines have been extensively studied during recent decades and, nowadays, recombinant human BMPs (rhBMPs) are widely used in several tissue-engineering products that might serve for the complete regeneration of bone or cartilage. Current applications include rhBMPs loaded in delivery systems made of synthetic or natural polymers and the differentiation of transplanted stem cells from the patient with rhBMPs for later body implantation. The purpose of this review is to cover the latest developments in the research for a BMP delivery carrier involving the

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use of biomaterials science, particularly with the use of natural origin polymers, to the recent preclinical trials and approved products for clinical applications.

2. Delivering BMPs

2.1. BMP carriers – from bench to clinical approval

The main role of a delivery system for BMPs is to retain these growth factors at the site of injury for a prolonged time frame, providing an initial support to which cells attach and form regenerated tissue (Seeherman and Wozney, 2005). The carrier should provoke optimal inflammatory responses, be biodegradable to allow the formation of an interface with the surrounding biological tissue or complete biodegradability for complete invasion of healed tissues, and present adequate porosity to allow the infiltration of cells and formation of blood vessels at the new bone. Furthermore, the carrier should protect the BMPs from degradation and maintain its bioactivity whilst releasing the protein in a time- and space-controlled way to promote the formation of new bone at the treatment site. Finally, carriers should be conveniently sterilizable, easy to handle, stable over time with well-defined storage procedures, as well as suitable for commercial production, allowing scale-up production and approval by regulatory agencies. The type of tissue to be regenerated is also of critical importance, as different mechanical requirements apply for the repair of bone, cartilage or tendon. For example, bone carriers are simplified by the fact that, upon fracture, bone is immobilized, but carriers should allow vascular ingrowth, due to the highly vascularized nature of bone. In cartilage, defects are subject to high compressive and shear stresses, thus making healing more challenging. In tendon, the regenerative ability appears to be intermediate between those of bone and cartilage, so tendons are very difficult to immobilize, needing a carrier that is able to withstand considerable tensile forces. The geometry of the carrier also significantly affects the biophysical process of osteoinduction and capillary penetration (Jin *et al.*, 2000). Taking all these factors into consideration, researchers also have to keep in mind that the carrier is evidently aimed for common usage by surgeons and physicians.

2.2. BMP retention at the orthopaedic site

The delivery of BMP should last for a sufficient period of time to induce a specific amount of bone mass to treat the defect. Retention of BMP at the orthopaedic site of injury is affected by many parameters, such as the interaction between the biomaterial and the BMP, and the influence of pH, temperature, porosity and the influence of salt concentrations. Evidently, retention of the growth factor depends on whether the BMP is immobilized on the carrier

during its manufacture or absorbed into the surface of the device.

Immobilization of the BMPs in a delivery system may be performed by different methodologies: via adsorption, entrapment or immobilization, or by covalent binding (Luginbuehl *et al.*, 2004). In case of adsorption, impregnation of the delivery matrix with the BMP is simpler but conformational changes might occur and the release of the protein be less sustained. Furthermore, delivery by adsorbed growth factors often results in initial burst release. With entrapment methodology, hydrophobic polymeric matrices are well known and described to immobilize and release bioactive agents over extended periods of time (Langer and Folkman, 1976). However, difficulty arises over the fact that during processing of certain materials into carriers, pH conditions or temperature conditions often result in denaturation of the protein. Much research nowadays aims to develop specific methods of producing delivery carriers for BMPs that do not cause their loss of activity. Lastly, the BMP may become immobilized by covalent binding to the carrier. This may be performed by production of a fusion BMP protein with a domain of specific binding to a biomaterial (Suzuki *et al.*, 2000). In this regard, recombinant technology offers great versatility for expression of a BMP capable of binding to most natural polymers. Other interesting approaches include exploring the strong affinity of BMPs to the extracellular matrix heparan sulphate/heparin proteoglycans (Blanquaert *et al.*, 1999), ion complexation by binding to charged polymers, as in the cases of chitosan, alginate, hyaluronans or synthetic polyelectrolytes (Yamamoto *et al.*, 1998), and crystallization of growth factors (Jen *et al.*, 2002).

2.3. Pharmacokinetic profiles of released BMPs

It is crucial to consider that release should preferably be sustained over time. Extremes in release profiles, such as long low-amount release of BMPs or initial burst of BMPs, are known not to be beneficial to bone healing. A delicate balance in the concentration of BMPs helps to prevent either insufficient binding to the carrier due to low concentration or precipitation due to high BMP concentration. The time of release may be dependent on the type of fracture or the application in question. It is clear that there is more than one desirable pharmacokinetic profile. The pharmacokinetic profile varies according to the material in consideration, its formulation and the type and amount of BMP in use. By chemical modification of the carrier or the BMP, we may achieve a specific release profile, which is of interest since different BMPs may present different release profiles due to their different amino acid sequences; and different species have different optimal release profiles (Li and Wozney, 2001) and the chances are that the optimal profile may be also site-specific. Depending on the site of injury or on a particular application, various formulations of delivery systems may be designed,

from simple nano/microparticles to scaffolds of increased three-dimensional (3D) complexity, such as those that mimic the physical properties of the extracellular matrix, or hydrogels that respond to physiological shifts such as pH or temperature.

3. Carrier BMPs

3.1. Synthetic biodegradable polymers

Synthetic polymers have been widely used in tissue-engineering applications (Saito and Takaoka, 2003) (See Table 1). Initially, poly(lactic acid) (PLA) was investigated as a carrier for BMP delivery (Miyamoto *et al.*, 1992) but the material was considered ineffective due the release of acidic degradation by-products. However, novel biodegradable synthetic polymers have attracted attention, since these are free of the risk of disease transmission that occurs with other materials used for bone applications, such as collagen. Biodegradable polymers, such as polylactic acid–*p*-dioxanone–polyethylene glycol (PLA–DX–PEG), allow percutaneous injection after heating, for use as a scaffold and a delivery carrier for BMPs, due to its versatile temperature-dependent liquid–semi-solid transition. This plasticity allows the biodegradation of the polymer to be synchronized with the induction of new bone by BMP (Saito *et al.*, 2001), and this type of injectable polymeric delivery system, polymerization

in situ, enables a less invasive approach to bone surgery (Saito *et al.*, 2003b). These scaffolds were tested, as carriers for BMPs, in a variety of models, such as a canine spinal fusion model and in the formation of artificial joints (Saito *et al.*, 2005b), for long bone defects in rabbits (Yoneda *et al.*, 2005) and in dogs (Murakami *et al.*, 2003), and in healing of rat cranial defects (Suzuki *et al.*, 2006). These studies showed that PLA–DX–PEG delivered rhBMP-2 successfully, inducing the repair of bone defects several weeks after implantation. In other reports, composites of PLA–DX–PEG with calcium phosphate were shown to require less rhBMP to induce new bone formation in mice (Matsushita *et al.*, 2004) and in healing femur defects of rabbits (Matsushita *et al.*, 2006). Composites of PLA–PEG with hydroxyapatite were also evaluated for articular cartilage repair in rabbits (Tamai *et al.*, 2005) and in a rabbit radii model (Kaito *et al.*, 2005), showing enhanced tissue repair in the animals treated with rhBMP-2 and hydroxyapatite composites.

Poly(lactic-co-glycolic acid) (PLGA) combines the adsorptive stability of PLA with the mechanical strength of polyglycolic acid (PGA) and has received particular attention (Winet and Hollinger, 1993). Biodegradation of the synthetic composite is achieved by varying the proportion of each of the two component materials (Miller *et al.*, 1977; Grayson *et al.*, 2004). PLGA as a carrier for rhBMP-2 delivery was reported in alveolar cleft repair in dogs (Mayer *et al.*, 1996), in gelatine sponge composites in a rabbit ulna model (Kokubo *et al.*, 2003),

Table 1. Synthetic polymer-based matrices/scaffolds for drug delivery of BMPs for tissue-engineering applications

Polymer(s)/carrier/scaffold structure	Formulation	Biological model	References
PLA	Scaffolds	Rabbit ulna	(He *et al.*, 2003)
	Scaffolds	*In vitro* differentiation of chondrocytes	(Yang *et al.*, 2006)
	Scaffolds	Rat ectopic bone formation	(Chang *et al.*, 2007)
PLA–collagen	Membrane	Rabbit ectopic bone formation	(Tian *et al.*, 2004)
PLA–collagen–HA	Composites	Radius defects in dogs	(Hu *et al.*, 2003)
	Composites	Mice ectopic bone formation	(Zhang *et al.*, 2005)
PLA–PEG–HA	Composites	Rabbit radius model	(Kaito *et al.*, 2005)
	Composites	Articular cartilage repair rabbits	(Tamai *et al.*, 2005)
PLA–DX–PEG	Scaffolds	Femoral canine model	(Murakami *et al.*, 2003)
	Scaffolds	Rat cranial defects	(Suzuki *et al.*, 2006)
	Scaffolds	Mice ectopic bone formation	(Kato *et al.*, 2006)
PLA–DX–PEG–CaP	Composites	Ectopic bone formation in mice	(Matsushita *et al.*, 2004)
	Composites	Spinal fusion in rabbits	(Namikawa *et al.*, 2005)
	Composites	Femur defects in rabbits	(Yoneda *et al.*, 2005)
	Composites	Femur defects in rabbits	(Matsushita *et al.*, 2006)
PGA	Membrane	Periodontal repair in dogs	(Wikesjo *et al.*, 2003)
	Scaffolds	*In vitro* cartilage formation	(Blunk *et al.*, 2003)
	Scaffolds	Cervical spinal fusion in goats	(Lippman *et al.*, 2004)
PLGA	Scaffolds	Alveolar cleft repair in dogs	(Mayer *et al.*, 1996)
	Scaffolds	Rabbit radius defects	(Hu *et al.*, 2005)
	Scaffolds	Alveolar ridge defects in rats	(Shimazu *et al.*, 2006)
	Scaffolds	Canine mandible defects	(Jones *et al.*, 2006)
	Scaffolds	Reconstruction of orbital floor defects in sheep	(Zheng *et al.*, 2006)
PLGA–heparin	Composite	Rat ectopic model	(Jeon *et al.*, 2007)
PLGA–gelatine	Composites	Rabbit ulna defects	(Kokubo *et al.*, 2003)
	Composites	Tooth defects in dogs	(Kawamoto *et al.*, 2003)
	Composites	Tibia defects in dogs	(Kokubo *et al.*, 2004)
PEG-based	Hydrogels	Rat cranial defects	(Lutolf *et al.*, 2003a, 2003b)
PEG-based, heparin	Hydrogels	Rat critical-sized calvarial defects	(Pratt *et al.*, 2004)
PEG-based	Hydrogels	Rat critical-sized calvarial defects	(Rizzi *et al.*, 2006)
Polypropylene fumarate	Hydrogels	Proliferation of chondrocytes	(Fisher *et al.*, 2004)
Isopropylacrylamide	Hydrogels	Ectopic bone formation	(Gao and Uludag, 2001)

in tooth defects of dogs (Kawamoto *et al.*, 2003) and in combination with bone marrow cells in a rabbit segmental bone defect model (Hu *et al.*, 2005). These studies confirm the good results that are usually obtained with PLGA scaffolds; bone formation was observed successfully when the scaffolds delivered rhBMP, as compared to controls. The dosage of rhBMP was also observed to significantly affect the repair of bone defects. Recently, PLGA scaffolds have been also tested in rats (Shimazu *et al.*, 2006), a canine model (Jones *et al.*, 2006) and sheep (Zheng *et al.*, 2006), showing that delivered BMP induced much higher bone formation than the scaffold alone over the several weeks following implantation. Another report, which involved a PLGA scaffold conjugated to heparin, showed that a much longer sustained release of rhBMP-2 and significantly increased *in vivo* new formation of bone were achieved (Jeon *et al.*, 2007), indicating the promising potential that heparin has as a stabilizing agent for BMP bioactivity.

Synthetic polymers have been also formulated as hydrogels for the delivery of BMPs. Since hydrogels contain large amounts of water, they are interesting devices for the delivery of therapeutic proteins. Lutolf *et al.* (2003a, 2003b) reported using synthetic PEG-based hydrogels that mimic the invasive characteristics of extracellular matrices, with integrin-binding sites for cell attachment and substrates for matrix metalloproteinases, in a rat model for rhBMP-2 delivery. The authors demonstrated that cells were able to fully penetrate the hydrogels and bone tissue was formed within 3–4 weeks in the gels that delivered rhBMP-2. Similarly, PEG-based hydrogels were reported by Pratt *et al.* (2004), showing that cells were able to fully invade the gel networks that were conjugated with peptides that mimic characteristics from extracellular matrix, such as plasmin and a heparin molecule to improve the rhBMP-2 stability. In another study, Fisher *et al.* (2004) evaluated thermoreversible hydrogels of poly(propylene fumarate–co-ethylene glycol) that mimicked properties of cartilage matrix hydrophilic proteoglycans, for cartilage tissue engineering, using rhBMP-7. The solutions of this polymer were aqueous at 25 °C but readily polymerized into gel above 35 °C. The group proposed the use of these hydrogels for articular cartilage repair. Identically, Gao and Uludag (2001) also reported using rhBMP-2 in *N*-isopropylacrylamide-based thermoreversible hydrogels in a rat model. The authors studied the effect of different hydrogel compositions on the *in vivo* retention of rhBMP and conclude that these polymers were very versatile for delivering proteins such as BMPs in more effective and controlled ways. A major disadvantage of the use of synthetic polymers is the risk of an inflammatory response, due to acidic by-products of degradation (Winet and Hollinger, 1993), which may be also detrimental to the stability of the incorporated BMPs. This has led researchers to look forward to other materials, such as collagen and other natural polymers, as alternatives for BMP delivery.

3.2. Collagen

Collagen is the major non-mineral component of bone and also the most abundant protein in connective tissues of mammals. Collagen has received much attention due to having good biocompatibility, degrading into physiologically compatible products and being suitable for interaction with cells and other macromolecules. The large variety of collagen formulations includes collagen gels, demineralized bone matrix, fibril collagen, collagen strips, membranes, absorbable collagen sponges and composites (Kirker-Head, 2000; Geiger *et al.*, 2003). Another advantage is that collagen can be processed in aqueous form. Collagen also has a favourable influence on cell infiltration and wound healing. During the last years, most researchers have focused on the use of absorbable collagen sponges, although several other formulations have been investigated (Kirker-Head, 2000). Collagen sponges are very versatile, easily manipulated and wettable. The manufacture of collagen sponge carrier depends on several factors, including sponge mass, cross-linking methods, sterilization methods, soaking time, protein concentration and buffer composition (Geiger *et al.*, 2003). These steps impact the interaction of the BMP with the collagen carrier and therefore the profile and the efficacy of released protein. For collagen sponges, binding of rhBMP is highly dependent on pH. Studies using modified versions of recombinant BMP led to the conclusion that modification of the isoelectric point could bring up to 100-fold differences in the retention of protein to the collagen carrier (Uludag *et al.*, 1999b). Binding of rhBMP-2 is therefore dependent on the isoelectric point of the two proteins and other factors, such as ionic strength. Collagen sponges have since been tested and evaluated in several animal models and clinical trials for cases of fracture repair, critical size defects, spinal fusion and dental and craniofacial reconstruction (Geiger *et al.*, 2003). The collagen sponge consists of lyophilized rhBMP, which is reconstituted with water prior to injection and impregnates the collagen sponge for several minutes before implantation. Two models using collagen sponges delivering recombinant human BMP-2 or BMP-7 were approved by the FDA for human use as an alternative to bone grafts, for spinal fusion and long bone fractures, after many pre-clinical trials that have been recently reviewed (Gautschi *et al.*, 2007). The collagen sponge holds the BMP and releases it only in the local environment where the surgery was performed, eliminating the need to harvest autologous bone, which causes post-operative pain. Based on the extensive preclinical and clinical trials, the use of collagen sponges delivering BMPs has been revealed to be a safer and superior alternative to autogenous bone grafting. However, although showing success, collagen sponges pose risks of immunogenic reactions, since the collagen used on these applications is derived from animal tissues, creating concerns about the risks of transmission of infectious agents and immunological reactions. For this reason, the development of a superior carrier material

Table 2. Natural origin polymer-based matrices for delivery of BMPs for tissue-engineering applications. Please refer to Table 3 for micro- and nanoparticle formulations

Polymer(s)/carrier	Formulation	Biological model	References
Alginate	Hydrogels	Ectopic bone formation in mice	(Simmons et al., 2004)
	Gels, synthetic BMP-2 oligopeptides	Ectopic bone formation and tibial defects in rats	(Suzuki et al., 2000; Saito et al., 2003a, 2004, 2005a)
	Gels	Rabbit radial bone defects	(Saito et al., 2006)
Carboxymethylchitosan	–	In vivo cartilage formation	(Mattioli-Belmonte et al., 1999)
Chitosan	Nanofibre membranes	Differentiation of osteoblast cells	(Park et al., 2006)
	Chitosan films	Differentiation of C2C12 cell line	(Lopez-Lacomba et al., 2006)
Chitosan–alginate	Gels	Trabecular bone formation in mice	(Park et al., 2005a)
Chitosan–gelatine	Composites	Differentiation of osteoblasts/myoblasts	(Liang et al., 2005)
Chitosan–PGA	Composites	*	(Hsieh et al., 2006)
Dextran	Hydrogels	Rat ectopic model	(Maire et al., 2005)
Fibrin	Sealant	Dental pulp of dogs	(Ren et al., 2000)
	Gels	Rats, rabbits, dogs and cats; different types of bone defects	(Schmoekel et al., 2004, 2005a, 2005b)
	Sealant	Rat calvarial defects	(Han et al., 2005)
	Sealant in PCL scaffolds	*	(Rai et al., 2005)
	Sealant	Ectopic bone formation in mice	(Zhu et al., 2006a, 2006b)
	–	Humans, frontal bone defect	(Arnander et al., 2006)
	Sealant	Differentiation of rabbit bone marrow cells	(Cui et al., 2007)
Fibrin–CaP	Sealant	Rat calvarial defects	(Hong et al., 2006)
Fibrin–collagen	Sealant in collagen sponge	Rat spinal model	(Patel et al., 2006)
Gelatine	Hydrogels	Rabbit skull defects	(Hong et al., 1998)
	Hydrogels	Ectopic bone formation in mice	(Yamamoto et al., 2001, 2003)
	Hydrogels	Critical-sized defects in rabbit ulnas	(Yamamoto et al., 2006)
	Hydrogels	Skull; non-human primates	(Takahashi et al., 2007)
Gelatine/dextran	Hydrogels	Differentiation on human periodontal ligament cells	(Chen et al., 2007a, 2007b)
Hyaluronic acid	Hydrogels	Ectopic bone formation in rats	(Bulpitt and Aeschlimann, 1999)
	Sponges	Alveolar ridge defects in dogs	(Hunt et al., 2001)
	Scaffolds	Differentiation of CH3H10T1/2 cells	(Kim and Valentini, 2002)
	–	Periodontal repair in dogs	(Wikesjo et al., 2003)
	Gels	Osteotomy in non-human primates	(Seeherman et al., 2004)
	Gels	Non-union tibial defects in rabbits	(Eckardt et al., 2005)
	Sponges	Rat mandibular defects	(Arosarena and Collins, 2005a, 2005b)
Hyaluronan acrylated	Hydrogels	Rat calvarial defects	(Kim et al., 2007)
Hyaluronic acid–Ti	Composites	Cranial defects in rats	(Itoh et al., 2001)
Hyaluronic acid–HA	Scaffolds	Osteointegration in cancellous bone in sheep	(Aebli et al., 2005)
Hyaluronic acid–PLA	Composites	Critical size defect in rat femurs	(Vogelin et al., 2005)
Silk fibroin	Films	Cranial defects in mice	(Karageorgiou et al., 2004)
	Scaffolds, loaded with human stem cells	Cranial defects in mice	(Karageorgiou et al., 2006)
	Electrospun nanofibres	Differentiation of human bone marrow cells	(Li et al., 2006)
	Scaffolds, loaded with human stem cells	Critical size femur defects in rats	(Kirker-Head et al., 2007)

*These studies involved solely material testing and delivery kinetics, with no in vitro or in vivo bioactivity models

for BMP delivery based on other natural polymers is currently being investigated. Alternatively, other sources of collagen, i.e. of recombinant origin, provides a means of obtaining reliable and chemically defined sources of purified human collagens that are free of animal components (Yang et al., 2004).

3.3. Natural origin polymers

The materials for tissue engineering applications should ideally mimic the natural environment of tissues and, in this regard, natural polymers can send signals to guide cells at the various stages of their development and thus accelerate healing (Mano and Reis, 2007). There are several natural polymers that may be used as carriers for BMP delivery (See Table 2). These include collagen, starch-based polymers, chitin and chitosan, hyaluronans, alginate, silk, agarose, soy- and alga-derived materials, and poly(hydroxyalkanoates) (Mano et al., 2007). Several of these polymers are derived from substances occurring in bone, cartilage or the extracellular matrix. For this reason, these materials often present excellent properties for use in regenerative medicine applications, such as being biodegradable, bioresorbable and versatile, as they may be processed into different formulations (Malafaya et al., 2003; Gomes et al., 2004). Natural polymers may present risks of immunogenic reactions and disease transmission, and disadvantages such as the sourcing and processing of the materials. Nevertheless, researchers have been looking for materials from plant origin and produced by microorganisms and/or from recombinant technology which may overcome these concerns.

Alginate is a generally safe polysaccharide, known to support the proliferation of chondrocytes *in vitro* (Park *et al*., 2005b). Very interesting work has been developed by Saito and colleagues with small synthetic peptides corresponding to BMP-2 regions binding to cell receptors, incorporated in cross-linked alginate gels, showing *in vitro* osteogenic differentiation and success in repairing bone defects in rats (Saito *et al*., 2003a, 2004, 2005a) and in rabbit radial bone defects (Saito *et al*., 2006). The use of alginate seems to be particularly appealing for cartilage tissue-engineering applications, since alginate is a major component of cartilage tissue. Alginate hydrogels were also reported for the delivery of rhBMP-2 in rats (Simmons *et al*., 2004) with the use of bone marrow cells with RGD peptide for improving cell adhesion.

Chitosan is another natural degradable polymer, obtained by alkaline deacetylation of chitin, extracted from the exoskeletons of arthoropods. Chitosan has been formulated in many forms, such as hydrogels (Baran *et al*., 2004) and fibre meshes (Tuzlakoglu *et al*., 2004), that showed potential for use in osteochondral tissue engineering, making it suitable for BMP delivery (Prabaharan and Mano, 2005). Several studies have reported the use of chitosan for delivering BMPs, particularly in composites with synthetic polymers or with other natural polymers. A chitosan–alginate composite gel, loaded with mesenchymal stem cells and rhBMP-2, was evaluated as an injectable tissue-engineering construct in mice and induced new trabecular bone formation over a period of 12 weeks (Park *et al*., 2005a). Liang and colleagues described a chitosan–gelatine scaffold with incorporated rhBMP-2 (Liang *et al*., 2005) which demonstrated increased expression of bone-marker osteocalcin in osteoblast and myoblast cell lines. In another report, a chitosan blend with PGA was studied as a novel delivery carrier for rhBMP-2 (Hsieh *et al*., 2006). Derivatives of chitosan are also reported. Chemical modification of chitosan may enhance certain bioactive properties and increase its solubility in water, thus aiding in the incorporation of rhBMPs, such as in the case of carboxymethyl chitosan. Mattioli-Belmonte *et al*. (1999) reported the use of *N,N*-dicarboxymethyl chitosan, with delivery of rhBMP, for enhancing cell proliferation and healing in articular cartilage lesions. Recently, rhBMP-2 was immobilized directly on a guided bone-regenerative membrane surface, made of chitosan nanofibres, that functioned as a bioactive surface to enhance bone-healing (Park *et al*., 2006). The BMP-2-conjugated membrane surface retained bioactivity for up to 4 weeks of incubation, as well as holding over 50% of the initial BMP-2 attached, promoting cell attachment, proliferation, ALP activity and calcification, when compared with BMP-2 absorbed to the membrane. In two other studies, dextran/gelatine-based microspheres, containing rhBMP-2, were adhered to chitosan films for guided-tissue regeneration (Chen *et al*., 2005a) and chitosan membranes activated with BMP-2 were also reported to successfully differentiate C2C12 cells (Lopez-Lacomba *et al*., 2006).

Fibrin is derived from blood cots and can be formulated into an adhesive glue-like delivery system (Hattori, 1990). Fibrin has been used as a delivery system for BMPs in a variety of animal models, including the use of a fibrin–fibronectin sealing system for rat calvarial defects as a carrier for rhBMP-4 (Han *et al*., 2005) and for rhBMP-2 (Hong *et al*., 2006), and a fibrin sealant with rhBMP-2 in the healing of dental pulp of dogs (Ren *et al*., 2000). In these reports, bone formation was much higher when the fibrin carrier was loaded with the rhBMP, as compared to controls. Fibrin glue might be also a great aid in limiting the diffusion of BMPs into the surrounding tissues, which could cause undesirable biological effects. In a rat spinal model, fibrin glue significantly limited the diffusion of rhBMP-2 that was loaded into a collagen sponge, preventing the BMP from inducing bone growth in the surrounding spinal cord and nerves (Patel *et al*., 2006). Interesting research has been developed by the Hubbell group with the use of fibrin matrices for the delivery of rhBMPs (Schmoekel *et al*., 2004, 2005a, 2005b). The group studied the influence of a non-glycosylated form of rhBMP-2 (Schmoekel *et al*., 2004) in fibrin. Since non-glycosylated rhBMP-2 is less soluble, retention into the fibrin scaffold was enhanced. The fibrin matrices were used to treat critical-size defects and non-unions in rats, dogs and cats. In these studies, bridging of bone defects showed more successful percentages of tissue healing when compared to controls. The group has also reported the use of a fusion BMP protein with an affinity domain to fibrin to increase binding to the carrier (Schmoekel *et al*., 2005b). Recently, a study using fibrin constructs to deliver rhBMP-2, vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2), combined with hyaluronic acid or collagen, dramatically improved the ability of blood vessels to directly invade the fibrin-based scaffolds (Smith *et al*., 2007). Finally, a human trial was reported showing partial reconstruction of a frontal bone defect using heparin together with bovine collagen, hyaluronic acid and fibrin as vehicles for rhBMP-2 (Arnander *et al*., 2006). Altogether, fibrin glue certainly seems to be a very useful addition to a bone tissue-engineering scaffold using BMPs, considering that it aids in promoting osteoinduction (Schwarz *et al*., 1993) and retention of growth factors (Hubbell, 2006).

Hyaluronans are present in the extracellular matrix and can be formulated into gels, sponges and pads. Hyaluronans have been used in a variety of trials as a delivery vehicle for rhBMPs, including in sponge form in the treatment of alveolar ridge defects in dogs (Hunt *et al*., 2001), periodontal repair in dogs (Wikesjo *et al*., 2003), tibial defects of rabbits (Eckardt *et al*., 2005), in sheep in combination with hydroxyapatite (Aebli *et al*., 2005), in the healing of critical size defect in rats in composites with polylactic acid (Vogelin *et al*., 2005), and in gel and paste forms in non-human primates (Seeherman *et al*., 2004). Kim and Valentini (2002) evaluated the kinetics of hyaluronic acid as a delivery system for rhBMP-2 *in vitro* and demonstrated that hyaluronan-based carriers retained more BMP than collagen gels. In two other

studies, hyaluronic acid was used to deliver BMPS for treating mandibular defecs of rats (Arosarena and Collins, 2005a, 2005b). Significantly more bone was formed in presence of rhBMP-2 and, although not significant, the volumes of new bone were larger for the hyaluronic acid carrier. Recently, a acrylated hyaluronic acid hydrogel was used with human mesenchymal stem cells and rhBMP-2 for healing of rat calvarial defects (Kim et al., 2007). Higher levels of osteocalcin expression and bone formation occurred when the BMP-2 and stem cells were tested. Diverse hydrogel formulations of hyaluronic acid were also evaluated by Bulppit and Aeschlimann (1999), showing excellent cell infiltration and osteochondral differentiation when loaded with BMP-2 in combination with either insulin growth factor-1 or transforming growth factor beta, implanted into rats. Hyaluronans are observed to interfere positively with BMP cascade (Zou et al., 2004) and, since these are part of the extracellular matrix, they may well be priority choices as scaffolds for the delivery of BMPs in regenerative medicine of bone.

Gelatine has been used mostly in form of hydrogels for delivery of BMPs. Gelatine is an irreversibly hydrolysed form derived from collagen that is usually cross-linked or hardened through thermal treatment to reduce its high water solubility and enhance the retention of protein to achieve a long-term release. Gelatine hydrogels delivering rhBMP-2 were studied in rabbit skulls (Hong et al., 1998), in mice (Yamamoto et al., 2003) and recently in the skulls of non-human primates (Takahashi et al., 2007). Gelatine hydrogels delivering rhBMP-2 were observed to show higher levels of ALP and osteocalcin in comparison with rhBMP-2 delivered in collagen sponges (Yamamoto et al., 2003). Recently, thermomechanical hydrogels based on methacrylated dextran in combination with gelatine have been reported by Chen et al. (2007a, 2007b). The group used rhBMP-2 encapsulated in microspheres of the same materials, loaded into the hydrogels, which delivered the growth factor over a period of 18–28 days. Their work is discussed below in the section on nanoparticles.

Dextran is another natural polysaccharide, synthesized by some bacteria, that has attracted attention for use as a BMP delivery system, because of its excellent hydrophilic nature and biocompatibility. Dextran has been particularly used in the form of nanospheres for delivery of rhBMPs, which is detailed in a later section. Dextran hydrogels has been evaluated for rhBMP-2 delivery, both *in vitro* and *in vivo*, in a rat ectopic model, showing formation of new bone (Maire et al., 2005). The possibility of using natural polymers for designing intelligent hydrogel systems for BMP delivery is also an interesting and very attractive option. However, no studies have been reported with the use of these systems.

Starch-based polymers are another interesting alternative for delivering BMPs that was proposed by Reis as materials with high potential for tissue engineering of bone and cartilage, due to their interesting mechanical properties (Malafaya et al., 2001; Elvira et al., 2002). These starch-based polymers are used in composites with different synthetic polymers and have been formulated into a variety of forms, such as hydrogels (Pereira et al., 1998), nanofibres (Tuzlakoglu et al., 2005), microparticles (Silva et al., 2004) or 3D scaffolds (Gomes et al., 2002). The wide variety of formulations and composites make these polymers suitable scaffolds for bone tissue engineering and controlled release of BMPs. In general, composites of natural polymers with synthetic polymers may become the future option of choice for bone tissue engineering, since they combine the specificities of synthetic and natural polymers to produce superior materials.

Silk fibroin is a protein derived from cocoons made by the larvae of silkworms. Silk has been proposed and widely investigated as a delivery carrier for BMPs in some contributions reported by the Kaplan group. In one study, rhBMP-2 was directly immobilized on silk fibroin films and the effect of the delivery system studied in human bone marrow stromal cells and in critical-sized cranial defects in mice (Karageorgiou et al., 2004). The rhBMP retained its biological activity. In another report, silk scaffold fibres, prepared by electrospinning, were used to deliver rhBMP-2 and hydroxyapatite nanoparticles for *in vitro* bone formation (Li et al., 2006). The rhBMP-2 survived the aqueous-based electrospinning process in bioactive form and induced osteogenesis in cultures of human mesenchymal stem cells. The group also tested BMP-2 delivered via silk fibroin scaffolds in critical size defects in mice (Karageorgiou et al., 2006). In both studies, the delivered rhBMP-2 increased levels of ALP activity and calcium deposition and transcript levels for bone sialoprotein, osteopontin, osteocalcin and runx2. In recent years Meinel and co-workers have evaluated the use of silk for tissue engineering constructs with silk–RGD covalently bound matrices, in human mesenchymal cells (Meinel et al., 2004), but not with use of BMPs. Meinel et al. (2006) tested human stem cells loaded in silk fibroin scaffolds, in combination with rhBMP-2, and compared stem cells transfected with BMP-2 via an adenovirus with exogenous protein. The expression of osteogenic markers was induced but the BMP was not studied when delivered directly on the silk scaffolds. Recently, rhBMP-2 delivered via silk fibroin scaffolds in combination with human mesenchymal stem cells was reported, with promising results, in the healing of critical-sized defects of femurs in rats (Kirker-Head et al., 2007). Compared with other protein-based materials, such as collagen, silks have distinguishable mechanical properties, presenting slower degradation times and thus allowing adequate time for proper bone remodelling. For this reason, silk is a feasible and potential option as a carrier for the controlled delivery of BMPs and, in general, for generating diverse bone tissue-engineering constructs for clinical applications (Meinel et al., 2005). Other possible sources of natural polymers for BMP delivery include soy, casein, polyhydroxyalkanoate, polyhydroxybutyrate, corals, carrageenan, gellan gum, agarose and other fibrous proteins, such as keratin and elastin (Kirker-Head et al., 2007).

3.4. Ceramics

Many studies have been dedicated to the understanding of the processes of bone mineralization and it was concluded that ceramic materials, such as hydroxyapatite (HA) and other types of calcium phosphates, can, when implanted, promote the formation of a bone-like mineral surface layer that leads to an increased interface between the materials and the surrounding bone. Calcium phosphate for tissue engineering of bone includes the use of calcium phosphate layers, films or coatings to promote bone ingrowth, and the use of calcium phosphate fillers to replace fractured or damaged bone. Hydroxyapatite (HA) is a form of calcium phosphate mineral that comprises 70% of bone and can be formulated as a powder, granules, disks or blocks (Tsuruga *et al.*, 1997). However, for bone tissue-engineering applications, specific formulations work better than others, dependent on the geometric structure of the carrier (Kuboki *et al.*, 1998). Hydroxyapatite is a fairly osteoconductive material, and has been used for BMP delivery alone (Noshi *et al.*, 2001) or in composites with natural or with synthetic polymers, as previously detailed. Hydroxyapatite has been used in combination with collagen for rabbit spinal fusion (Kraiwattanapong *et al.*, 2005), with natural origin polymers (Aebli *et al.*, 2005), with tricalcium phosphate in a rabbit calvarial model delivering rhBMP-2 (Schopper *et al.*, 2007), for differentiating mesenchymal stem cells with BMP-14/GDF-5 (Shimaoka *et al.*, 2004), or for lumbar spinal fusion in non-human primates (Boden *et al.*, 1999). Based on these and other studies, hydroxyapatite has proved to be a suitable carrier for BMP delivery, not only enhancing the delivery of the growth factor but also in aiding its retention to the carrier and the osteoconductivity of the scaffold (Uludag *et al.*, 1999a).

Calcium phosphates for delivery of BMPs include calcium phosphate (Ca-P) cements and ceramics and calcium phosphate coatings. Ca-P cements have been extensively investigated, as they are osteoconductive, biocompatible and show fast deposition of new bone at the cement surface (Driessens *et al.*, 1998). The BMP may be incorporated into low-temperature Ca-P cements by adding the protein in lyophilized form, or in aqueous phase prior to formation of cement, without any risk of denaturation of the growth factor. In high-temperature cements, the BMP is generally only adsorbed onto the surface. A porous structure can be fabricated to mimic the structure of trabecular bone (Dutta Roy *et al.*, 2003). Trials for rhBMP-2 delivery have included studies in rabbit ulnas (He *et al.*, 2003) and femurs (Cao *et al.*, 2006) and a canine tibial defect model (Edwards *et al.*, 2004). These studies have demonstrated that the use of Ca-P cements accelerates bone healing. Trials are have also demonstrated the efficacy of calcium phosphate matrices in some non-human primates trials, such as in alveolar ridge surgery using a composite of Ca-P, hydroxyapatite and a collagen sponge (Miranda *et al.*, 2005), in osteotomy sites with a single percutaneous injection of rhBMP-2 loaded

into Ca-P cements (Seeherman *et al.*, 2006a), and in posterolateral fusion, where Ca-P functioned as a bulking agent to improve the osteogenic potential of rhBMP-2 loaded onto an absorbable collagen sponge (Barnes *et al.*, 2005). Seeherman *et al.* (2006b) also reported achieving bridging of critical-sized defects in rabbits using the same minimally invasive injectable Ca-P cements. Ruhé *et al.* (2005, 2006) also reported several *in vivo* studies with the use of calcium phosphate cements loaded with rhBMP-2. A main advantage of the use of calcium phosphates compared to other carriers is that, in general, high doses of rhBMPs are not required (Yuan *et al.*, 2001).

Calcium phosphate coatings are another elegant approach for delivering BMPs, by incorporating these growth factors into the lattice-work of these mineral layers that may be used to coat specific scaffold materials. The BMP is biomimetically deposited during the formation of the calcium phosphate film that is formed when the material is immersed in a solution of simulated body fluid that mimics the human blood plasma (Liu *et al.*, 2004). The de Groot group has used calcium phosphate-coated titanium disks for delivery of rhBMP-2 in a rat model, showing that much lower concentrations of BMP are required in comparison with collagen matrices (Liu *et al.*, 2004, 2005). Alternatively, bioactive glass (45S5 – Bioglass®), a synthetic surface reactive glass that is commonly used as a filler for damaged or fractured bone, may be also used to form biomimetic calcium phosphate-coated scaffolds (Leonor *et al.*, 2003). The biomimetic layers, similar to bone apatite, may be used in combination with BMPs to guide the attachment and differentiation of bone precursor cells, given that the coatings have been shown to promote osteointegration and osteoinduction. Silva *et al.* (2004) proposed using blends of starch with polylactic acid and bioglass microspheres for the delivery of BMPs. Promising potential arises from the fact that bioglass is osteoconductive and osteoinductive, stimulating the recruitment and differentiation of osteoblasts, which produce new bone and completely resorb the material.

3.5. Microparticles and nanoparticles for BMP delivery

The search for efficient, simple and cheap delivery systems for drug targeting has led to great investment in the area of nanoparticles and microparticles for drug delivery. Most common materials for the design of nanodevices to deliver BMPs include synthetic materials, natural polymers and hydroxyapatite-based particles. Both nano-scale (up to 100 nm) and microspheres are reported (See Table 3).

Polylactic acid and polylactic-co-glycolic acid have been used as materials for nanoparticle-based delivery systems for BMPs. PLA was initially studied as a carrier for BMPs in a rat ectopic bone formation model (Saitoh *et al.*, 1994), showing formation of new bone at 4 weeks after implantation and mature bone after 24 weeks. However, by blending PLA with polyglycolic

Table 3. Micro and nanoscale drug delivery systems based on synthetic and natural-origin polymers. The average size or size range of the particles is noted on the formulation

Carrier	Formulation/size	Biological models	References
PLGA	Microparticles	Rat calvarial bone defects	(Kenley et al., 1994)
	Microparticles (247–430 µm)	Rat femurs	(Lee et al., 1994)
	Microparticles	In vitro differentiation of osteoblasts	(Oldham et al., 2000)
	Microparticles	Rabbit calvarial bone defects	(Schrier et al., 2001)
	Microparticles	Sheep vertebrae	(Phillips et al., 2006)
	Nanoparticles (300 nm)	Rat ectopic model	(Wei et al., 2007)
PLGA/Ca-P	Microparticles (66 µm)	Rat ectopic model/cranial model	(Ruhe et al., 2005)
PLA	Microparticles	Rat ectopic bone formation	(Saitoh et al., 1994)
Collagen–HA	Microparticles	Rabbit femoral bone defects	(Wang et al., 2003)
	Nanocrystals/fibres	Dogs, spinal fusion/tibial fractures	(Itoh et al., 2004)
Chitosan–alginate	Microparticles	In vitro differentiation of rabbit bone marrow stem cells	(Qin et al., 2003)
Dextran	Nanoparticles (20 nm)	In vitro differentiation of rabbit bone marrow stem cells	(Chen et al., 2005b)
Dextran–PEG	Microparticles (20–40 µm)	In vitro differentiation of human periodontal ligament cells	(Chen et al., 2006)
Dextran–gelatine	Microparticles (20–40 µm)	Canine defects	(Chen et al., 2005a)
	Microparticles (0.5–1.5 µm)	Periodontal regeneration in dogs	(Chen et al., 2007b)

acid in copolymer polylactic-co-glycolic acid (PLGA), biodegradation is controlled by changing the proportions of each of the two materials, since PLA degrades much more slowly than PGA. Microspheres of PLGA have since then been evaluated in diverse animal models, such as in rat calvarial bone defects (Kenley et al., 1994), rat femurs (Lee et al., 1994) and in calvarial defects in rabbits (Schrier et al., 2001), forming much more bone when BMPs were delivered via the PLGA particles. Interesting work has been developed by Ruhé and colleagues, using microspheres of PLGA in combination with Ca-P cement, as carriers for rhBMP-2 delivery (Ruhe et al., 2005). The release of rhBMP-2 was observed to be dependent on composite composition and nanostructure, as well as on the pH of the release medium. Sustained slow release was observed, possibly due to the interaction of rhBMP-2 with the calcium phosphate cement. Delivery of rhBMP-7 was evaluated in PLGA nanospheres encapsulated in PLA scaffolds, with interconnected macroporous and nano-fibrous architectures (Wei et al., 2007). The group concluded that the carrier delivered rhBMP-7 in a time-controlled manner and induced significant bone formation.

Diverse natural origin materials were also proposed as carriers at a nano- and micro-scale for delivering BMPs. Collagen–hydroxyapatite microspheres were evaluated for rhBMP-4 delivery in femoral defects of rabbits (Wang et al., 2003). Regeneration occurred in the animal group treated with BMP-4 particles, while with the carrier alone the defects were filled with fibrous tissue and inflammatory cells. Microspheres based on blends of chitosan with sodium alginate were reported in vitro in bone marrow-derived cells, showing an increased in the levels of ALP (Qin et al., 2003). During recent years, dextran-based microspheres and nanospheres were extensively evaluated by Chen and colleagues for the delivery of BMPs. In 2005, the group reported delivering BMP-2 with dextran-based microparticles (20–40 µm) in canine defects (Chen et al., 2005a) and nanoparticles (20 nm) in the differentiation of rabbit bone marrow cells (Chen et al., 2005b). One year later, the authors studied a

novel class of methacrylate dextran–PEG microspheres in periodontal ligament cells (Chen et al., 2006). Recently, the group reported dextran–gelatine microspheres loaded into thermomechanical dextran/gelatine hydrogels to deliver rhBMP-2 for periodontal regeneration in dogs (Chen et al., 2007b). The group studied the kinetics of release and demonstrated that, by changing the ratio of components, the rhBMP release could be varied from 18 to more than 28 days (Chen et al., 2007a).

Nanoparticle technology seems definitely one of the most promising approaches for the future of bone tissue engineering, by overcoming some fundamental issues in the methods applied for tissue regeneration such as the insufficient mechanical strength of scaffolds and the lack of stability or bioactivity of growth factors such as BMPs at the defect site (Kim and Fisher, 2007). Major nanotechnology areas of research, such as the fabrication of scaffold–nanoparticle composites and the design of nano-patterned materials, are some of the areas we found with the greatest potential for the delivery of BMPs in orthopaedic regenerative science.

4. Human clinics and the future of bone tissue engineering

In the Western world, an estimated 5–10% of all bone fractures show deficient healing, leading to delayed union or non-union, causing significant morbidity and psychological stress to the patients and bringing elevated costs to society (Westerhuis et al., 2005). Fortunately, the current advances in bone tissue engineering have led researchers to find new strategies and devices with the use of BMPs for accelerating the healing of bone tissues in the orthopaedic field. In fact, by the end of 2007, nearly 1 million patients worldwide were projected to have been treated with BMPs for diverse bone-related problems and diseases (Pecina and Vukicevic, 2007). The clinical uses of BMPs include spinal fusion, treatment of long bone defects and non-unions, dental and periodontal tissue engineering, craniofacial defects and diseases, fracture

repair, the improvement of osteointegration with metallic implants, musculoskeletal reconstructive surgery and tendon and ligament reconstruction. There are currently two main collagen-based products containing BMP-2 or BMP-7 that were approved by the FDA in recent years for human clinical use: Infuse™ Bone Graft (Medtronik, US; Wyeth, UK), containing rhBMP-2, and Osigraft™ (Stryker Biotech), containing rhBMP-7, known by the designation of OP-1 (osteogenic protein-1). BMP-2 Infuse™ bone graft was approved for certain interbody fusion procedures in 2002, for open tibial fractures in 2004, and for alveolar ridge and sinus augmentations in 2007 (McKay *et al.*, 2007). BMP-7 Osigraft™ was approved for long bone fractures and as an alternative to autografts in patients requiring posterolateral lumbar spinal fusion. There has been also an increasing number of trials that provide supporting evidence for the use of rhBMP-7/OP-1 in the treatment of open tibial fractures, distal tibial fractures, tibial non-unions, scaphoid non-unions and atrophic long bone non-unions (White *et al.*, 2007).

4.1. Spinal fusion

Spinal fusion applications are an important part of currently ongoing clinical trials (Carlisle and Fischgrund, 2005). Spinal fusions consist of nearly half of all grafting surgery. Furthermore, failure rates of up to 35% have been reported. The interest is in the use of rhBMPs to accelerate healing in patients with disk degenerative disease, removing the need for autograft harvesting and reducing morbidity. Degenerative disc disease is defined as back pain caused by degeneration of the discs, as confirmed by clinical data and symptoms. The common approach is to use a collagen or other carriers soaked with rhBMP and place these within titanium spacers called cages which are implanted into the spine. There are two types of fusion approaches: posterolateral fusion, involving placing the bone graft between the transverse processes in the back of the spine, and interbody fusion, which involves placing the bone graft between the vertebrae in the area occupied by the intervertebral disk. In interbody fusion of lumbar vertebrae, based in the success of previous trials, a prospective study for rhBMP-2 was performed by McKay and Sandhu (2002) involving 279 patients with disk degenerative disease, from which 143 patients received tapered lumbar cages filled with rhBMP-2 and 136 patients received the device filled with autologous bone from iliac crest. Since, at the conclusion of the study, higher rates of spinal fusion were observed for the rhBMP-2 group and less operative time and morbidity were reported, the FDA granted approval for the use of rhBMP-2 in the treatment of single-level lumbar degenerative disc disease. Posterolateral spinal fusions are common for treating spondylolisthesis but require distinct mechanical and biological properties of the carrier. Boden *et al.* (2002), using rhBMP-2 delivered via a biphasic carrier of tricalcium phosphate and hydroxyapatite, reported achieving complete fusion in all patients treated with

rhBMP-2 as compared with the control group. Although the carrier did not obtain approval from FDA, there are several other trials currently ongoing in humans, such as with collagen sponges (Glassman *et al.*, 2007).

The efficacy of use of rhBMP-7/OP-1 as a replacement for iliac crest autograft was first evaluated by Johnsson *et al.* (2002), showing higher rates of fusion in posterolateral spinal fusions with application of rhBMP-7, which were confirmed by further studies (Vaccaro *et al.*, 2004; Kanayama *et al.*, 2006). With no adverse effects, OP-1 was considered a viable alternative to autograft and, as a result, FDA gave approval of rhBMP-7 for patients who have failed a posterolateral fusion and are at risk for repeated pseudarthrosis. At present there is little focus on human trials involving either the use of other BMPs or the use of natural polymers as delivery carriers, but is to be expected that these options will be soon evaluated clinically, in the next few years, considering the current state of biomaterials research.

4.2. Long bone fractures

In most cases where rhBMPs are applied to fractures, these consist in non-unions of long bones. It is estimated as an example that in the UK, 42% of these fractures are of tibias, 20% are femurs and the rest are of other bones (Giannoudis and Tzioupis, 2005). RhBMP-2 has received FDA approval for use in treating open tibial fractures. Initially, Govender *et al.* (2002) performed a randomized trial with 450 patients having open tibial fractures. The patients were randomized to receive different doses of rhBMP-2, 0.75 mg/ml (total dose of 6 mg), 1.50 mg/ml (total dose of 12 mg) or no rhBMP-2, in collagen sponges. After 12 months, analysis showed accelerated healing and reduced infection with increasing dosing of rhBMP-2. There are numerous studies in the literature suggesting that rhBMP-7/OP-1 is also a safe and effective alternative for the treatment of diverse long-bone fractures and non-unions. In tibial non-unions, a trial that led to multiple regulatory approvals worldwide concluded that OP-1 delivered in a collagen sponge was a safe and effective alternative to bone grafting (Friedlaender *et al.*, 2001). In scaphoid non-unions, Bilic *et al.* (2006) concluded that OP-1 could allow successful use of allograft, eliminating the donor site morbidity of an iliac crest autograft. In another curious trial, McKee and colleagues assessed the efficacy of OP-1 on treating diverse long bone non-unions on 62 patients that failed previous autograft operations. The bones involved included 16 tibiae, 18 clavicles, 11 humeri, 10 femora, four ulnae and three radii. At the end of the study, 54 of 61 non-unions (89%) had healed, indicating that rhBMP-7 was an effective treatment (White *et al.*, 2007).

4.3. Dental tissue engineering

In periodontal and dental tissue engineering, rhBMPs find their place in inducing pulp stem cells to differentiate

into odontoblasts and promoting the regeneration of pulp and teeth. Since the pulp is an organ known to have tremendous regenerative abilities, during recent years tissue engineering has been considered as a promising approach for diverse clinical cases, such as caries, pulpitis and apical periodontitis (Nakashima and Akamine, 2005). Particularly interesting is the fact that human pulp stem cells have self-renewal ability and that tubular dentine is formed after the transplantation of these stem cells with hydroxyapatite power in mice (Gronthos *et al.*, 2002). Recombinant BMPs have been noted to induce dentine formation *in vivo* when delivered with a collagen scaffold (Nakashima, 1994). The ultimate goal in dental tissue engineering using BMPs is in achieving a complete restoration of the physiological, structural and mechanical integrity of the native dentine–pulp complex, including nerve and vascular regeneration (Nakashima and Akamine, 2005).

4.4. Future challenges, a global perspective

Bone repair and regeneration with BMPs are ushering in a new era in orthopaedics. The past 10 years have seen practical demonstration of bone repair in a series of animal studies and subsequently in clinical trials. The expected value of BMPs in the treatment of bone defects, spinal fusion applications and other types of related applications is enormous. Extensive research in preclinical models has led to the approval of restricted use for human trials. However, despite the significant evidence of potential for bone healing demonstrated in animal models, future clinical investigations will be needed to better define variables such as dose, scaffold and route of administration. The impressive results of animal models are difficult to replicate in humans. It is unclear why these differences occur. Some insight is provided by the clear species-specific dose response, ranging from 25 µg/ml in rodents to 50 µg/ml in dogs, 100 µg/ml in non-human primates and 800 µg/ml in humans (Luginbuehl *et al.*, 2004). The recruitment of bone precursor cells and bone turnover may occur differently in rodents, small animals and large mammals. Likewise, the dosing may not yet be optimal. In fact, the concentrations of BMP in use are supraphysiological and a million times higher (milligrams in assays as compared to the nanogram range *in vivo*). BMP inhibitors such as noggin or sclerotin, which are upregulated by BMP presence, may be interfering and providing a negative feedback effect on the bodily healing mechanisms (Westerhuis *et al.*, 2005). Understanding the regulation between BMPs and BMP-inhibitors might be a key issue. Moreover, different fractures may require different dosages (Schmitt *et al.*, 1999). Critical issues to consider include the potential risk of BMPs inducing heterotopic bone formation, especially when implanted adjacent to neural tissues (Paramore *et al.*, 1999), and the serious issue of reported antibody formation, noted in up to 38% of patients in some trials with BMPs (Walker and Wright, 2002).

Clearly, the use of BMPs in orthopaedics is still in its early days, but the latest trials in humans suggest that an exciting and promising future will unfold in the development of novel tissue-engineering products for a wide range of clinical situations, with the use of BMPs. To date, clinical trials have focused mostly on rhBMP-2 and -7 and with the use of collagen as delivery materials. However, given the intricate network of molecules interplaying during bone regeneration, it is possible that a 'cocktail' of different BMPs with simultaneous or sequential release would be the most desirable approach to clinical uses, instead of a single stimulus or molecule (Hadjiargyrou *et al.*, 2002). Raiche and Puleo (2004a, 2004b) have already explored the sequential release of rhBMP-2 in combination with insulin-like growth factor-1 (IGF-1). However, the development of such a cocktail for clinical cases may encounter difficulties, since the commercial rights of the two currently approved rhBMPs are for restricted use and owned by different companies (Westerhuis *et al.*, 2005). Nevertheless, in the near future, the emergent advances with recombinant production of BMPs (Klosch *et al.*, 2005; Schmoekel *et al.*, 2005b; Bessa *et al.*, 2007) will aid researchers in obtaining larger amounts of bioactive rhBMPs which could be used for tissue-engineering research and the development of novel products.

With the excitement over the potential of other natural-origin polymers as novel delivery systems for BMPs, there is little doubt that these will also find relevant places in regenerative medicine of bone and traumatology, and may be soon approaching clinical trials in humans. Diverse natural-origin polymers have shown promising success for bone tissue engineering, such as fibrin, hyaluronic acid, chitosan, silk fibroin and starch-based composites. Furthermore, these overcome limitations and disadvantages from the use of synthetic polymers and the risks of disease transmission inherent to the use of collagen from bovine sources. The recent advances in biomaterials science will certainly boost the number of tissue-engineering approaches for the healing of bone with the use of BMPs. Novel strategies will possibly involve the specific targeting of BMPs, in injectable systems and stimulus-responsive hydrogels, the use of nano-scale patterning or encapsulated particles, or with the use of molecules combined with the BMP, mimicking the extracellular matrix, all of which allow restricted and site-specific delivery of these growth factors. Additionally, the design of 3D specific-architecture scaffolds by methods such as rapid prototyping or the design of bilayered scaffolds surely ensures that the carrier for delivering the BMP will closely mimic the bone structure. Guided tissue-engineering delivery systems, which would deliver not only BMPs but also angiogenic factors, would, for instance, prompt the recruitment and distribution of blood vessel precursor cells, which is necessary for the formation of mature bone. Finally, the use of Ca-P cements and biomimetic coatings is a very promising approach, since it furthers mimics the bone mineral make-up and aids in retaining the BMP and improving

tissue–material integration. The expanding variety of options for biomedical use of BMPs gives the promise that the future of clinical regenerative medicine. and that of BMPs, particularly for bone applications, will be certainly be a bright one in the coming decades for millions of people.

Acknowledgements

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