

Review

Bone Regeneration Using Bone Morphogenetic Proteins and Various Biomaterial Carriers

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Abstract: Trauma and disease frequently result in fractures or critical sized bone defects and their management at times necessitates bone grafting. The process of bone healing or regeneration involves intricate network of molecules including bone morphogenetic proteins (BMPs). BMPs belong to a larger superfamily of proteins and are very promising and intensively studied for in the enhancement of bone healing. More than 20 types of BMPs have been identified but only a subset of BMPs can induce *de novo* bone formation. Many research groups have shown that BMPs can induce differentiation of mesenchymal stem cells and stem cells into osteogenic cells which are capable of producing bone. This review introduces BMPs and discusses current advances in preclinical and clinical application of utilizing various biomaterial carriers for local delivery of BMPs to enhance bone regeneration.

Keywords: bone morphogenetic proteins; BMPs; bone regeneration; biomaterials; carrier methods

1. Introduction

Trauma, tumor resection, and disease frequently result in fractures or critical sized bone defects. As they do not usually heal spontaneously, their management necessitates bone grafting or major surgical reconstruction [1]. About 5%–10% of all these procedures are associated with impaired healing, which results in psychological stress and morbidity to patients and incur significant economic cost to society. According to the American Academy of Orthopaedic Surgeons more than 6.3 million people suffer from bone fractures annually in the U.S alone [2]. The management of around 25% of those requires some sort of bone grafting [3,4]. Many of these procedures involve the use of autogenous bone that is commonly harvested from the iliac crest [5]. Recent studies have demonstrated that harvesting iliac crest bone grafts is associated with increased risk of morbidity [6–8]. It has also been shown that two years following the iliac crest bone graft surgery, up to 25% patients may still feel donor site pain [9]. This potential morbidity together with the limited procurement of autogenous bone has long been the driving force for scientists to develop improved bone graft substitutes [10].

Bone graft substitutes can be broadly categorized into two major types [9]. The first are the osteoconductive materials. These are essentially bone void fillers that allow bone in-growth and are usually made of bioresorbable materials. Common examples include collagen composites, sea-coral, and various ceramics [4,9,11]. Based on the size of defect, these can be used alone or in conjunction with autogenous bone as they are deemed ineffective as a sole treatment for critical sized bone defects [9]. The second category of bone graft substitutes are referred to as the osteoinductive materials, which comprise substitutes that contain biological factors, such as growth factors. These factors can recruit progenitor cells, induce their differentiation into bone forming cells (osteoblasts) and form bone even in a non-osseous site [9]. Because of their tremendous potential to heal and regenerate lost tissue, many osteoinductive materials are currently being investigated for various tissue engineering applications.

Over the years the advent of tissue engineering has been seen as a promising alternate to the current standard of care and can potentially circumvent many limitations encountered with conventional autogenous grafts involving additional surgical procedures [12]. Tissue engineering utilizes the patient's own precursor cells, matrices, and growth factors to regenerate the lost tissues. Since the early promise shown by research in this field, bone regeneration has received much more interest, as bone is one of the tissues with highest regenerative potential in human body [13]. Bone regeneration can be considered as recapitulation of embryonic bone development because bone heals via generation of new bone instead of scar tissue formation [14]. This process of bone healing or regeneration involves intricate network of molecules including bone morphogenetic proteins (BMPs) [15]. BMPs are the very promising as well as the most intensively studied group of growth factors that are involved in the enhancement of bone healing [16–18]. Ever since Urist's discovery of BMPs as bone inducing proteins, interest in tissue engineering of bone for orthopaedic, craniofacial and periodontal applications has increased exponentially [13,17,19–25]. Many research groups have shown that BMPs can induce differentiation of mesenchymal stem cells and stem cells into osteogenic cells which are capable of producing bone [26–28]. BMPs and their various types are introduced and a discussion of current advances in preclinical and clinical application of various biomaterial carriers for local delivery of BMPs to enhance bone regeneration is presented in this review.

2. Structure of BMPs

BMPs belong to a larger superfamily of proteins referred to as transforming growth factors beta (TGF- β) superfamily [15]. The TGF- β superfamily can be broadly categorized into TGFs, BMPs (excluding BMP-I which is a proteinase and a member of tolloid like proteins), growth factors 1–10, which are considered a subclass of BMPs, Vg related genes, glial derived neurotropic factor, inhibins, activins, nodal related genes, and drosophila genes [29]. BMPs are produced as large precursor proteins which undergo disulphide bond dimerization before they are proteolytically cleaved at consensus Arg-X-X-Arg site, yielding mature dimers [30]. Studies have revealed that stability of the processed mature protein is controlled by N-terminal region and efficiency of cleavage is determined by downstream sequence adjacent to cleavage site [26,31]. It has also been suggested that this enzymatic cleavage takes place prior to secretion of BMPs [30]. Following secretion, BMPs can bind to two classes of transmembrane receptors (type 1 and type 2) that are known to have serine threonine kinase activity [30,32,33]. Ligand binding is required for type 1 receptor kinase activation; whereas, activity of type 2 receptor kinase is constitutive. However, optimal ligand binding requires presence of both type 1 and type 2 receptors [30]. Once ligand attaches to type 2 receptor, it transphosphorylates type 1 receptor which leads to activation of type 1 kinase. This, in turn, leads to phosphorylation of members of Smad (protein) family of transcription factors, which are then translocated to the nucleus where subsequent expression of target genes takes place [30,34].

To date, more than 20 types of BMPs have been identified (Table 1) [30,35] but it has been shown that only a subset of BMPs can induce de novo bone formation [14]. Although the mechanism by which BMPs induce osteoblastic differentiation still remains to be elucidated, it is known that these growth factors play a pivotal role in regulation of osteoblastic differentiation [36]. A substantial body of evidence suggests that BMPs like BMP-2, BMP-7, and BMP-9 can provide primordial signal for differentiation of osteoprogenitor cells into osteoblasts which then form the bone extracellular matrix [37–42]. Preclinical studies have shown that recombinant human forms of BMPs, especially BMP-2, BMP-4 and BMP-7 can regenerate lost tissue when used with an adequate carrier in critical sized bone defects [36,43,44]. Promising data from these preclinical studies together with encouraging results from clinical trials have found the basis for approval of rhBMP-2 and rhBMP-7 for clinical use by FDA [36,45]. These growth factors have been studied extensively during the last two decades and different recombinant human BMPs (rhBMPs) are currently being investigated for their potential use in several tissue engineered products which may lead to complete regeneration of bone [13,21,46–57]. Currently available BMP based applications include BMP loaded synthetic or natural delivery systems and BMP induced differentiation of patient's transplanted stem cells for later body implantation [13,58–60].

Table 1. Types, organs of expression and functions of BMPs.

BMP Type	Human Chromosome	Expression in Human Tissues	Functions Performed in Humans
BMP-1	8p21.3	Heart, Skeletal Muscle, Kidney, Lung, Liver, Pancreas, Prostate, Bone Marrow, Thymus, Spleen, Spinal Cord, Brain	Metalloprotease that cleaves COOH–propeptides of procollagens I, II, and III/Induces cartilage formation/ Cleaves BMP antagonist chordin [61]
BMP-2	20p12	Pancreas, Spleen, Kidney, Lung	Skeletal repair and regeneration/ Heart formation [62,63]
BMP-3 (osteogenin)	4q21.21	Bone Marrow, Spleen, Brain, Heart, Prostate, Pancreas, Skeletal Muscle, Thymus,	Negative regulator of bone Morphogenesis [64]
BMP-3b (GDF10)	10q11.22	Spinal Cord, Skeletal, Muscle, Prostate, Brain, Pancreas	Cell differentiation regulation/ Skeletal morphogenesis[65]
BMP-4 (BMP-2b)	14q22–q23	Brain, Spinal Cord, Spleen, Thymus, Bone Marrow, Heart, Skeletal Muscle, Kidney, Lung, Liver, Prostate, Pancreas	Skeletal repair and regeneration/ Kidney formation [66–68]
BMP-5	6p12.1	Prostate, Spleen, Thymus, Bone Marrow, Brain, Spinal cord, Pancreas, Lung, Heart, Skeletal Muscle, Kidney	Limb development/ Bone and cartilage morphogenesis/ Connecting soft tissues [69,70]
BMP-6 (Vrg1, Dvr6)	6p24–p23	Bone Marrow, Thymus, Heart, Skeletal Muscle, Spleen, Brain, Spinal Cord, Muscle, Kidney, Lung, Liver, Prostate, Pancreas	Cartilage hypertrophy/ Bone morphogenesis/ Nervous system development [71,72]
BMP-7 (OP1)	20q13	Brain, Spinal Cord, Prostate, Thymus, Bone Marrow, Spleen, Heart, Skeletal Muscle, Kidney, Lung, Liver, Pancreas	Skeletal repair and regeneration/ Kidney and eye formation/ Nervous system development [72–75]
BMP-8a (OP2)	1p34.3	Pancreas, Heart, Kidney, Thymus, Bone Marrow, Spleen, Brain, Spinal Cord, Lung, Prostate	Bone morphogenesis/ Spermatogenesis [76]
BMP-8b	1p35–p32	Brain, Spinal Cord, Heart, Bone Marrow, Pancreas, Spleen, Skeletal Muscle, Kidney, Liver	Spermatogenesis [76]
BMP-9 (GDF2)	10q11.22	Liver	Bone morphogenesis/Development of cholinergic neurons/Glucose metabolism/Anti-angiogenesis [77]
BMP-10	2p13.3	Thymus, Spleen, Brain, Spinal Cord, Heart, Lung, Pancreas, Prostate, Bone Marrow, Skeletal Muscle, Liver	Cardiac morphogenesis [77]
BMP-11 (GDF11)	12q13.2	Thymus, Bone Marrow, Pancreas, Spinal Cord, Brain, Spleen	Patterning mesodermal and Neural tissues, Dentin Formation [78]
BMP-12 (GDF7/CDMP2)	2p24.1	Data not available	Ligament and Tendon development/ Sensory neuron development [79]
BMP-13 (GDF6/CDMP2)	8q22.1	Data not available	Normal formation of bones and joins/Skeletal morphogenesis/ Chondrogenesis [80,81]
BMP-14 (GDF5/CDMP1)	20q11.2	Bone Marrow, Kidney, Liver, Heart	Skeletal repair and regeneration [75]
BMP-15 (GDF9b)	Xp11.2	Data not available	Oocyte and Follicular development [82]
BMP-16	Data not available	Data not available	Skeletal repair and regeneration [83]
BMP-17	Data not available	Data not available	Data not found [84]
BMP-18	Data not available	Data not available	Data not found [84]

Biological function, structure, physiology, signaling pathways and regulation of BMPs expression have already been reviewed in detail elsewhere [15,26,29,30,85,86]. In the present review we discuss the promising results obtained from the use of BMPs based tissue engineered bone constructs from preclinical experiments as well as clinical studies and the significant strides that have been made in recent years in the field of BMPs carriers.

3. Carriers for BMPs

Although it has been shown that BMPs can initiate bone formation in solution form [87], the dose required to induce bone formation can be dramatically reduced if BMPs are implanted with an appropriate carrier [87]. The principle role of BMP carrier system is to retain these growth factors at the site of injury for prolonged period of time and render an initial support for cells to attach and elaborate the extracellular matrix with subsequent regeneration of lost architecture [13]. There are a variety of biomaterials that can and have been investigated for BMP delivery for bone tissue engineering applications (Table 2). A carrier should ideally induce optimal inflammatory response, should be completely biodegradable and present adequate porosity for infiltration and proliferation of cells and sprouting blood vessels at the site of new bone formation [13]. Moreover, it should prevent degradation of BMPs while maintaining their bioactivity and allow a sustained release in a controlled manner to promote new bone formation at the site of defect [13,18,88–90]. Lastly, it should be easily sterilized, easy to handle and stable when stored and be commercially feasible allowing scale up production. In this section we discuss various carriers that have shown great promise and can potentially be used to construct an efficient and effective BMPs based tissue engineered bone construct.

Table 2. Biomaterial carriers investigated for BMP delivery in bone tissue engineering.

Carrier	BMP	Matrix type	Model
Synthetic Polymers			
PGA	BMP-2	Membrane	Dog periodontal repair [91]
	BMP-2	Scaffolds	Cervical spinal fusion in goats [92]
	BMP-9	Scaffolds	<i>In vitro</i> bone/cartilage formation [93]
PLGA	BMP-2	Scaffolds	Orbital floor reconstruction in sheep [94]
	BMP-2	Scaffolds	Rabbit femoral head necrosis [95]
	BMP-2	Scaffolds	<i>In vitro</i> release and rat calvaria [96]
	BMP-2	Scaffolds	Canine mandibular defects [97]
	BMP-2	Scaffolds	Alveolar ridge defects in rats [98]
	BMP-2	Scaffolds	Rabbit radius defects [99]
	BMP-2	Scaffolds	Alveolar cleft repair in dogs [100]
PLGA-gelatin	BMP-2	Composites	Rabbit ulnar defects [101]
	BMP-2	Hydrogel/scaffold	Rabbit knee cartilage/bone interface [102]
	BMP-2	Composites	Dog tooth defects [103]
PLGA-fibrin	BMP-2	Composites	Dog tibia defects [101]
	BMP-2	Sealant	Rabbit radial bone defect [104]
PLGA-heparin	BMP-2	Composites	Ectopic model in rat [105]
PLA	BMP-2	Scaffolds	Rabbit ulna [106]
	BMP-2	Composite	Radial defects in rabbit [107]
	BMP-2	Scaffolds	Ectopic bone formation in rats [108]

Table 2. Cont.

Carrier	BMP	Matrix type	Model
PLA-collagen	BMP-2	Membrane	Ectopic bone formation in rabbits [109]
	BMP-2	Composite	Rat ectopic bone formation [110]
PLA-DX-PEG	BMP-2	Scaffolds	Femoral canine model [111]
	BMP-2	Scaffolds	Rat cranial defects [112]
	BMP-2	Scaffolds	Mice ectopic bone formation [113]
PEG-based	BMP-2	Hydrogels	Rat cranial defects [114,115]
	BMP-2	Hydrogels	Rat critical sized cranial defects [116]
	Bmp-2	Hydrogels	<i>In vitro</i> release profiling [117]
PEG-based, heparin	BMP-2	Hydrogels	Rat critical sized calvarial defects [118]
Isopropylacrylamide	BMP-2	Hydrogels	Ectopic bone formation [119]
Natural Polymers			
Fibrin	BMP-2	Gels	Rabbits, dogs, rats and cats; various bone defects [120–122]
	BMP-2	Sealant	Ectopic bone formation in mice [123,124]
	BMP-2	Sealant	Human frontal bone defect [125]
	BMP-2	Sealant	Differentiation of rabbit bone marrow cells [126]
Fibrin-collagen	BMP-2	Sealant in sponge	Rat spinal model [127]
Fibrin-heparin-collagen	BMP-2	Sponge	Mouse calvarial defects [128]
Fibrin-heparin	BMP-2	Sponge	Spinal fusion in rabbit [129]
	BMP-2	Sponge	Posterior lumbar fusion in rabbits [130]
Gelatine	BMP-2	Hydrogel	Rabbit skull defects [131]
	BMP-2	Hydrogel	Non-human primate skulls [132]
	BMP-2	Hydrogel	Ectopic bone formation in mice [133,134]
Hyaluronic acid	BMP-2	Hydrogel	Ectopic bone formation in rats [135]
	BMP-2	Hydrogel	<i>In vitro</i> release model [136]
	BMP-2 & 4	Sponges	Rat mandibular defects [137,138]
	BMP-2	Sponges	Dog alveolar ridge defects [139]
	BMP-2	Scaffolds	Periodontal repair in dogs [91]
	BMP-2	Gels	Osteotomy in non-human primates [140]
	BMP-2	Gels	Non-union tibial defects in rabbits [141]
Hyaluronic acid -PLA	BMP-2	Composite	Rat femurs critical sized defects [142]
Silk fibroin	BMP-2	Films	Cranial defects in mice [143,144]
	BMP-2	Nano-fibers (electrospun)	Differentiation of human bone marrow cells [145]
	BMP-2	Scaffolds	Critical sized defects in rats [143]
	BMP-2	Scaffolds	Cranial defects in mice [146]
Alginate	BMP-2	Hydrogels	Ectopic bone formation in mice [147]
	BMP-2	Gels	Tibial defects in rats and ectopic bone formation [112,148,149]
	BMP-2	Gels	Rabbit radial bone defects [150]
Chitosan	BMP-2	films	C2C12 cell line differentiation [151]
	BMP-2	Membranes	Osteoblast cell differentiation [152]
Chitosan-collagen	BMP-7	Scaffold	Cell differentiation [153]
Chitosan-alginate	BMP-2	Gel	Mice trabecular bone formation [154]
Chitosan-gelatine	BMP-2	Composite	Osteoblast differentiation [155]
Dextran	BMP-2	Hydrogel	Rat ectopic model [156]

Table 2. Cont.

Carrier	BMP	Matrix type	Model
Titanium			
Titanium	BMP-2	Implant (porous)	Dog humerus[157]
	BMP-2	Implant (porous)	Dog mandible [158]
	BMP-7	Implant (threaded)	Rabbit femur [159]
	BMP-2	Particles	<i>In vitro</i> assay [160]
	BMP-2	Shell capsule composite	Alveolar bone reconstruction [161]
Titanium-HA	BMP-2	Cylinder	Sheep tibia [162]
	BMP-2	Implant coating	<i>In vitro</i> evaluation [163]
Titanium-HA-heparin	BMP-2	Composite	<i>In vitro</i> and <i>in vivo</i> (distal femur of rabbit) [164]
Titanium-chitosan	BMP-2	Composite	<i>In vitro</i> model [165]
Micro and Nanoscale Carriers & Polymer-Ceramic Composites			
PLGA	BMP-7	Microparticles	Sheep vertebrae [166]
	BMP-2	Microparticles	Rabbit calvarial bone defects [167]
	BMP-2	Microparticles	Osteoblast differentiation <i>in vitro</i> [168]
	BMP-2	Microparticles	Rat femurs [169]
	BMP-2	Microparticles	Rat calvarial bone defects [170]
PLGA-CaP	BMP-2	Microparticles	Rat cranial and ectopic model [171,172]
PLA	BMP-2	Microparticles	Ectopic bone formation in rats [173]
PLA-PCL	BMP-2	Nanoparticles	Radius of rabbits [174]
Collagen-HA	BMP-4	Microparticles	Rabbit femoral bone defects [175]
	BMP-2	Scaffold	Implantation in rat hind limb [176]
	BMP-2	Scaffold	<i>In vitro</i> release study [177]
	BMP-2	Nanocrystals/fibres	Spinal fusion, tibial fractures in dogs [178]
Dextran	BMP-2	Nanoparticles	<i>In vitro</i> differentiation of rabbit bone marrow cells [179]
Dextran-PEG	BMP-2	Microparticles	<i>In vitro</i> differentiation of rabbit bone marrow cells [180]
Dextran-gelatin	BMP-2	Microparticles	Periodontal regeneration in dogs [181]
Chitosan-alginate	BMP-2	Microparticles	Canine defects [182]
Hyaluronic acid-HA	BMP-2	Composite	Osteointegration in sheep cancellous bone [183]
PLA-collagen-HA	BMP-2	Composite	Mice ectopic bone formation [184]
	BMP-2	Composite	Radius defects in dogs [185]
PLA-PEG-HA	BMP-2	Composite	Rabbit radius model [186]
PLA-DX-PEG-CaP	BMP-2	Composite	Femur defects in rabbits [187]
	BMP-2	Composite	Femur defects in rabbits [188]
	BMP-2	Composite	Spinal fusion in rabbits [189]
Fibrin-CaP	BMP-2	Sealant	Rat calvarial defects [190]
CaP	BMP-2	Scaffold (porous)	Maxillary sinus floor elevation in rabbits [191]
	BMP-2	Solid free form fabricated scaffold	<i>In vitro</i> and <i>in vivo</i> evaluation [192]
Biphasic CaP	BMP-7	Scaffold	Ectopic mouse model [193]
HA-TCP	BMP-2	Scaffold	Rabbit calvarium [194]
	BMP-2	Scaffold	Ectopic bone formation in rats [195]

Notes: PGA: Poly-glycolic acid; PLGA: Poly-lactic-glycolic acid; PLA: Poly-lactic-acid; DX: Dioxanone; PEG: Poly-ethylene-glycol; HA: Hydroxylapatite; Ca-P: Calcium phosphate; PCL: Polycaprolactone.

3.1. Ceramics

Research has shown that ceramics such as hydroxyapatite and other types of calcium phosphate materials can promote formation of bone like mineral surface leading to increased interface between bone and the implanted material [196]. Hydroxyapatite (HA), which comprises about 70% of bone, is an osteoconductive [197,198] material that can be formulated as blocks, disks, powder or granules [199]. Various research groups have tested HA alone [200] or in combination with other polymers for delivery of BMPs [194,201,202]. These studies demonstrate that HA is a very promising carrier for delivery of BMPs not only because it is osteoconductive and aids in retention of growth factors but also because it enhances the delivery of growth factors [47,203–205]. HA has very low biodegradation and that is a major disadvantage [196,206]. This limits the amount of newly formed bone that can replace the resorbing graft tissue [206]. To overcome this problem, β -TCP can be added to HA, to create a biphasic calcium phosphate composite material [207]. This has higher resorption rate and well described bioactivity [208,209].

Being osteoconductive and biocompatible, calcium phosphate based ceramics, cements and coatings have also been studied extensively. Association of BMP into a bone-like calcium phosphate could possibly help to control the release of BMP [210]. A major advantage in using calcium phosphate as rhBMPs carrier in comparison to other materials lies in the fact that high doses of rhBMPs are not required for bone formation [13,47,211]. Various studies have shown that rhBMP-2 when delivered through calcium phosphate based delivery systems results in accelerated bone healing [212,213]. Similarly studies on non-human primates have also yielded promising results [214,215]. Calcium phosphate based BMPs delivery systems have tremendous potential for tissue engineering based bone constructs but clinical trials need to be carried out to determine their effectiveness before they can be routinely used as an alternate to autogenous bone grafting procedures.

3.2. Non-Ceramics

3.2.1. Synthetic Biodegradable Polymers

Various synthetic polymers have been used extensively in tissue engineering applications [13,216–218]. The possibility of prevention of disease transmission in grafting procedures through use of synthetic polymers instigated the scientists to develop synthetic polymer based BMP carriers. Initially Polylactic acid (PLA) due to its adsorptive stability was investigated as a potential BMP carrier in the early 1990s [219], but was soon considered ineffective due to release of acidic degradation by products [220]. Further research lead to development of a new generation of PLA-based synthetic polymers, including polylactic acid-p-dioxanone-polyethylene glycol (PLA-DX-PEG) and polylactic acid-polyethylene glycol (PLA-PEG) [216,221,222]. Due to its versatile temperature dependent liquid-semi solid transition, PLA-PEG allows percutaneous injection after heating [13]. This injectable approach provides a less invasive alternative to open surgical procedure [218]. Similarly experiments with PLA-DX-PEG showed promising results. It was observed that synchronization existed between new bone formation by BMP and polymer biodegradation [223]. PLA-DX-PEG has been tested by different research groups in various animal models [59,187,222,224]. Further research has tested composites of PLA-DX-PEG with calcium phosphate and demonstrated that combination of calcium phosphate with PLA-DX-PEG

reduces the requirement of rhBMP needed to induce new bone formation [13,188,225]. Recently developed composites of hydroxyapatite with PLA-PEG, hydroxyapatite with polyamide and hydroxyapatite with collagen composites have also shown great promise when used in conjunction with rhBMP-2 for tissue repair in different animal models [186,226,227].

Polyglycolic acid (PGA) which has superior mechanical strength when combined with PLA results in Poly(lactic-co-glycolic acid) (PLGA) which has received much attention for tissue engineering applications [228]. Biodegradation of the composite can be controlled by changing the proportion of the two materials [229,230]. PLGA has been tested in various studies and the promising results show tremendous potential of PLGA as a carrier for BMPs [99–101,103]. Interestingly when rhBMP was used in conjunction with PLGA, much higher bone formation was observed in comparison to PLGA alone, highlighting the osteoinductive potential of BMPs [94,97,98]. More recently a new approach involving conjugation of heparin to PLGA scaffold was tested by Jeon and co-workers [105]. They reported that the resultant composite demonstrated a much longer sustained release of rhBMP-2, resulting in significantly more new bone formation [105].

As hydrogels contain large amounts of water, they have long been considered potential candidates for proteins and drug delivery [231–235] and many synthetic polymers have already been formulated as hydrogels for BMPs delivery. PEG based hydrogel with extracellular matrix-like characteristics, such as integrin binding sites for cellular attachment and substrates for matrix metalloproteinases (MMPs) for delivery of rhBMP-2 has been reported [114,115]. These studies showed promising results with initial cellular penetration followed by bone tissue formation within the hydrogel [114,115]. In a similar study, Pratt and co-workers [118] demonstrated cellular penetration of PEG-based hydrogel, which was conjugated with heparin and plasmin for improving rhBMP-2 stability [118]. Similarly Fisher and colleagues reported successful use of poly-propylene fumarate-co-ethylene glycol based rhBMP-7 carrying thermo-reversible hydrogel for tissue engineering of cartilage [236]. The authors concluded that these hydrogels could be potentially used for regeneration of cartilage tissue [236]. In another study, Gao and Uludag [119] demonstrated that N-isopropylacrylamide and N-acryloxysuccinimide based hydrogels could be successfully used for effective and controlled delivery of proteins such as BMPs [119]. The main drawback of using synthetic polymers is the risk of potential inflammatory response due to acidic by-products because of polymer degradation [228] which may interfere with the stability of adsorbed BMPs. This has incited scientists to look for other materials that can serve as BMP delivery carriers without such limitations.

3.2.2. Natural Polymers

Ideally an implant based on the principles of tissue engineering should mimic natural environment of tissues and in this context natural polymers can render the additional benefit of accelerated healing as they can send signals to guide cells in various stages of their development [13,176,237]. Various natural polymers including collagen, silk, alginate, agarose, chitin and chitosan have been tested as potential carriers for delivery of BMPs [238]. Many of these materials are developed from substances naturally present in extracellular matrix, cartilage and bone. Therefore, it is no surprise that these materials exhibit excellent properties for potential use in regenerative medicine [239,240].

In a series of studies, Saito and co-workers developed alginate gels incorporated with peptides corresponding to BMP-2 region that binds to cell receptors. Using this gel they demonstrated successful repair of bone defects in rat and rabbit models [149,150,241]. Simmion and co-workers [147] also reported successful delivery of rhBMP-2 in rats using alginate hydrogels [147]. Chitosan derived from alkaline deacetylation of chitin has also been formulated in different forms including fibre meshes [242] and hydrogels [243] which have shown great promise for use in tissue engineering of bone and cartilage making it a potential candidate for delivery of BMPs [13,244]. In a study by Park *et al.* [154], the authors demonstrated that a composite gel comprising of chitosan and alginate loaded with rhBMP-2 and mesenchymal stem cells could induce new bone formation [154]. In another *in vitro* study, Liang and co-workers observed that when rhBMP-2 was incorporated in a chitosan-gelatin based scaffold, it increased the expression of osteocalcin, a biomarker of osteoblast cell lines [155]. It has been shown that chitosan and PGA, and chitosan and collagen based composite scaffold for delivery of rhBMP-2 has tremendous potential in bone regenerative therapies due to enhanced release amount and sustained release of rhBMP-2 [153,245].

Fibrin, which can be formulated in an adhesive glue like delivery system [246] has also been studied as a potential carrier for BMPs delivery in different animal models. It has been tested *in vivo* as a carrier for rhBMP-4 [247] and rhBMP-2 [190] in the form of fibrin-fibronectin sealing system and for rhBMP-2 [248] in the form of fibrin sealant. These studies demonstrated much higher bone formation in test sites where fibrin carrier was loaded with rhBMP as compared to control sites. Other research groups have also reported development of fibrin matrices for delivery of rhBMPs [120–122]. These matrices were used to treat bone defects in rat, cats and dogs. They reported bridging of the bone defect with functional bone healing, demonstrating effectiveness of this delivery system. All in all, fibrin-based BMPs carriers are a valuable addition to bone engineering scaffolds considering they promote bone formation [249] and allow retention of growth factors [250].

Hyaluronans distributed widely throughout the connective tissue can also be formulated into pads, sponges and gels for delivery of rhBMPs. In a study by Kim and Valentini [251], the authors demonstrated that hyaluronan based rhBMP-2 carrier retained higher concentration of BMP in comparison to collagen gels [251]. Since then hyaluronan based carriers have been used in a number of studies to deliver rhBMP [139–142,183,252]. More recently hyaluronic acid based carrier was used to deliver BMPs for treatment of mandibular defects in rats. The authors found that significantly more bone was formed when rhBMP-2 was used in addition to carrier in comparison to carrier alone [137,138].

Silk has also been suggested as a possible carrier for BMPs delivery. Derived from silkworm larvae cocoons, silk has been extensively investigated by various research groups for use as a BMPs vehicle [13]. Following *in vitro* and *in vivo* studies, Karageorgiou and colleagues reported that rhBMP-2 retained its activity when it was directly immobilized on silk fibroin films [144]. In another study, silk-based scaffold was used to deliver rhBMP-2 [145]. The authors reported that this delivery system induced osteogenesis in cultures of mesenchymal stem cells with increase in alkaline phosphatase activity and calcium deposition [145]. Similar results were obtained when silk fibroin scaffold was used to deliver BMP-2 at bone defect sites in mice [143]. Others have also reported promising results when rhBMP-2 in combination with human mesenchymal stem cells delivered through silk fibroin scaffold were used in treatment of critical sized bone defects in rats [146]. In comparison to other protein-based materials, degradation rate of silk is slower which allows sufficient time for bone healing. This particular advantage

makes silk a very promising candidate for delivery of BMPs and for development of various bone tissue engineering constructs [253].

Collagen is the most abundant protein in mammalian connective tissue and is the main non-mineral component of bone. It can also positively influence cellular infiltration and wound healing. Another advantage lies in its ability to be processed in aqueous form [13]. Furthermore collagen and its breakdown products are also physiologically biocompatible [13,153] and, hence, it is no surprise that many collagenous formulations including demineralised bone matrix, collagen strips, resorbable collagen sponges, collagen gels, and fibril collagen have been prepared for applications in tissue engineering [51,153,176,254–257].

Versatility, wettability and ease of manipulation has led many scientists to test the possibility of use of collagen sponges as a carrier for delivery of rhBMPs in various tissue engineering applications including fractures and critical sized bone defects [176,258]. Numerous studies have revealed safety and effectiveness of collagen sponge and two collagen sponge delivery systems have been approved by FDA for delivery of rhBMP-2 and rhBMP-7 as an alternate to bone grafts for spinal fusion and long bone fractures [258–260]. However, the collagen used in these carriers is of animal origin and poses a risk of immunological reaction and possibility of transmission of infectious agents and diseases [261,262] and hence scientists are constantly striving to develop a superior delivery system for BMPs.

3.2.3. Titanium

BMPs were first tested in 1994 for surgical reconstructions in craniomaxillofacial surgery using titanium implants [263]. Titanium implants treated with BMP-2 [264] have also been tested along with bioactive titanium dioxide/hydroxyapatite surfaces functionalized with BMP-2 *in vitro* [163,265]. *In vivo* testing of bone response to titanium implants with BMP has also been evaluated [266]. In a sheep model, the osteointegration of hydroxyapatite-titanium implants coated with non-glycosylated BMP-2 was evaluated and showed promising bone response [162]. Osteoblast differentiation and mineralization promoted by a globular fibrinogen layer through cell autonomous BMP signaling on titanium carrier surfaces has been studied [54]. Greater bone formation was demonstrated on apatite-coated titanium with incorporated BMP-2/heparin *in vivo* [267]. The effect of immobilization of heparin and BMP-2 to titanium surfaces has been studied for improving osteoblast function and osteointegration [169,268–271]. Surface modification of titanium with hydroxyapatite-heparin-BMP-2 has been shown to enhance the efficacy of bone formation and osseointegration *in vitro* and *in vivo* [164]. Fabrication of printed titanium shells for containment of BMP-2 composite graft materials for alveolar bone reconstruction has also been researched upon [161].

3.3. Microspheres, Nanoparticles and Ceramic/Polymeric Composite Microspheres

Over the years great deals of resources have been invested in the area of micro and nanoparticles in search of simple, efficient and cheap drug delivery systems. Researchers have also tested microspheres and nanoparticles for delivery of BMPs [13]. Following promising results of PLGA based BMPs delivery systems, microspheres of PLGA have been studied in various animal models including calvarial bone defects in rats [170], rat femur [272] and rabbit calvarial defects [167]. These studies demonstrated that presence of rhBMP within PLGA microspheres was necessary for bone formation [272] and resulted in

restoration of normal contouring and radiopacity of defects whereas only soft tissue formation was observed when PLGA microspheres were used alone [170].

Wang and colleagues have also evaluated collagen-hydroxyapatite composite microspheres for delivery of BMPs. They observed that when BMP-4 based particles were implanted in rabbit femoral defects, significant bone formation took place in comparison to influx of inflammatory cells and fibrous tissue formation at sites, which were treated with carrier particles alone [175]. Recently Chen and co-workers carried out a series of interesting studies where they used dextran based microspheres and nanoparticles for delivery of BMPs [273,274]. They reported that by varying proportion of the constituent components, the release of rhBMP could be increased to more than 28 days [275]. Novel approaches that use nanoparticles of sulphated chitosan, hydroxylapatite, silica, metallofullerene have also been explored to deliver BMPs for bone tissue engineering applications [53,57,276–283]. Although there are some unresolved issues in use of microspheres or nanoparticles for delivery of BMPs like inadequate mechanical strength of scaffold or loss of bioactivity of growth factor [284] but nanoparticle technology is one of the most promising approaches for future of tissue engineering of bone.

4. Bone Regeneration Using BMPs

4.1. Preclinical Studies

Research has revealed that BMPs play a critical role in growth and differentiation of various cell lines including osteoblasts [15]. A number of preclinical experiments including animal studies have demonstrated the effectiveness of recombinant human BMPs in regeneration of bone [27,285–294]. Many of these preclinical studies used critical sized bone defect model. In bone, “critical sized defects” are defined as defects that do not heal without intervention [295]. For instance healing of critical sized bone defects by BMP-2 was shown in different species including rabbits, sheep, dogs and non-human primates [17,85]. Healing of bone defects using genetic approach where an implant comprising of a bioresorbable polymer mixed with mesenchymal stem cells transfected with adenovirus BMP-2 has also been reported [85]. It has also been shown that systemic administration of rhBMP-2 results in increased activity of mesenchymal stem cells and reversal of age related and ovariectomy induced bone loss [296]. Recently different research groups have also shown that rhBMP-2 when delivered on a calcium phosphate carrier or with liposome carrier, results in accelerated bone healing in rat and rabbit models [85]. In another study complete bone regeneration was observed when rhBMP-2 soaked collagen was grafted in critical sized calvarial defects in rats [297]. Similar results were observed by Yasko and co-workers following grafting of rhBMP-2 in rat femoral defects [298]. In another study, femoral defects in sheep showed evidence of new bone formation four weeks post rhBMP-2 grafting. Eight weeks later complete bone union was verified by radiographical analysis. Histological evaluation after 52 weeks of implantation revealed presence of woven and lamellar bone [299]. A study in dogs evaluating the role of rhBMP-2 in bone defects revealed complete healing of mandibular defects within three months. The authors then assessed the bone quality by degree of mineralization, bone thickness and biomechanical strength over the three months. They observed significant improvement in all three parameters [300]. In a series of recent studies, Cook and colleagues demonstrated that grafting of collagen based rhBMP-7 particles resulted in restoration of critical sized bone defects in rabbits and dogs. Radiographical evidence of complete

union was observed at the end of two months. Biomechanical experiments showed that mean torsional strength of the unions was comparable to that observed in intact bone [285,301]. In a study in non-human primates by Ripamonti and co-workers, evidence of new bone formation was observed as early as 15 days post-surgery and complete bone formation was achieved in three months [302]. Similar results were obtained when rhBMP-7 was grafted in sinuses and dental extraction sites in chimpanzees [303]. All these studies suggest that BMPs can lead to complete healing of critical sized bone defects in short period of time in various species.

4.2. Clinical Studies

It is surprising that despite the positive role BMPs play in accelerating fracture repair and bone healing [18,22,304–308], they have been studied only to a limited extent in human clinical trials. However, in the last decade many clinical studies were conducted which yielded promising and encouraging as summarized in (Table 3).

Table 3. Clinical studies carried out using BMPs for bone tissue engineering.

Clinical Studies Using BMP-2			
Authors	Type of Fracture	Methods	Findings
Herford, A.S. and Boyne, P.J. [309]	Mandibular Continuity Defect	Patients were treated with rhBMP-2 alone or in conjunction with collagen carrier without concomitant bone material.	Successful osseous restoration of critical sized edentulous area was observed which was then followed by prosthetic treatment.
Sweeny, L., Lancaster, W.P., Dean, N.R., Magnuson, J.S., Carroll, W.R., Louis, P.J., Rosenthal, E.L.[310]	Mandible	Test Group: Standard treatment plus rhBMP-2. Control Group: Standard treatment without use of rhBMP-7.	There was no significant difference in the measured outcomes between the two groups.
Govender, S., <i>et al</i> [311]	Open Tibial Shaft Fractures	Control Group: Received standard of care. Test Group: Received standard of care with implant containing rhBMP-2 in concentration of 0.75 mg/mL or 1.5 mg/mL.	The implant containing 1.5 mg/mL rhBMP-2 was significantly superior to standard of care in accelerating fracture and wound healing, reducing of rate of infections and frequency of secondary interventions. It also reduced the overall invasiveness of the procedure.
Tressler, M.A., Richards, J.E., Sofianos, D., Comrie, F.K., Kregor, P.J., Obremesky, W.T.[312]	Long Bone Non-unions	Patients were given standard treatment with iliac crest bone graft or rhBMP-2.	No statistically significant difference was observed in rate of healing and postoperative infection. Iliac bone graft resulted in significantly more intraoperative blood loss and longer operative procedures.
Bibbo, C., Patel, D.V., Haskell, M.D. [313]	High risk ankle and hind foot fusion	Patients were treated with standard of care in conjunction with rhBMP-2.	Successful union was achieved in 96% fracture sites. The authors concluded that rhBMP-2 is an effective adjunct for treatment of high risk ankle and hind foot fusions.

Table 3. Cont.

Clinical Studies Using BMP-7			
Authors	Type of Fracture	Methods	Findings
Moghaddam, A., Elleser, C., Biglari, B., Wentzensen, A., Zimmermann, G. [314]	Long Bone Non-unions	Patients who had atrophic non-union of long bones were treated with rhBMP-7 in a Type 1 Collagen carrier without concomitant bone graft material, with bone graft and bone graft and osteosynthesis revision.	Successful union was observed in 82% fractures. Healing was confirmed clinically as well as radiographically.
Dohin, B., Dahan-Oliel, N., Fassier, F., Hamdy, R. [315]	Persistent non-union involving different bones	OP-1 (BMP-7) with Type 1 Collagen carrier was used in conjunction with standard of care.	Clinical and radiographical evidence of bone healing was observed in 74% patients. The authors concluded that OP-1 stimulates healing of persistent non-union without serious adverse effects.
Moghaddam-Alvandi, A., Zimmermann, G., Büchler, A., Elleser, C., Biglari, B., Grützner, P.A., Wöfl, C.G. [316]	Non-union in Long Bones	rhBMP-7 was applied in non-union fracture of long bones. Before application of rhBMP-7 patients had already underwent surgical treatment an average of 3.3 times.	Proper bone healing was observed in 92% fracture sites. The authors concluded that although rhBMP-7 may not be used in all non-union cases, it appears to be effective in treatment of complex cases.
Nicodemo, A., Capella, M., Deregibus, M., Massè, A. [317]	Non-union Sacral Fracture	Patients who had previously failed to respond to standard of care were treated with rhBMP-7 as an adjunct to standard of care.	The use of rhBMP-7 resulted in successful healing of fractures which had previously failed to heal with traditional surgical techniques.

A study by Govender and co-workers [311] showed that when rhBMP-2 was delivered using an absorbable collagen sponge for treatment of open tibial fractures, there was a 44% reduction in risk of failure of healing. The authors also reported significantly less secondary invasive interventions and overall shorter healing time in comparison to control group [311]. This combination of rhBMP-2 and absorbable collagen has been approved by Food and Drug Administration (FDA) and regulation authorities in Europe and is being commercialized under the name of InFuse in the US and InductOs in Europe. In a similar study, rhBMP-7 bound to bovine type 1 collagen was compared to autogenous bone graft for treatment of non-union tibial fractures. Although there was no improved healing with rhBMP-7 but the study showed that results with rhBMP-7 and bovine type 1 collagen were comparable to autogenous bone graft, which is considered the gold standard in treatment of critical sized bone defects [318]. In another study by Canadian Orthopaedic Trauma Society, the use of rhBMP-7 for treatment of open tibial shaft fractures was evaluated. Patients were randomly divided into test groups which received rhBMP-7 and control groups where rhBMP-7 was not part of the treatment modality. Clinical, radiological and serological testing revealed that a significantly larger number of patients in the test group were able to fully bear weight without pain at the 12 month follow up period in comparison to the control group. Secondary intervention for delayed union and non-union was also significantly lower in the rhBMP-7 group as compared to the control group. Furthermore, no rhBMP-7 related adverse effects were encountered [319].

In a similar study, use of rhBMP-7 was evaluated for treatment of distal tibial fractures [320]. Patients treated with hybrid external fixation and BMP-7 was compared with patients who received similar treatment without the use of rhBMP-7. Authors reported that the mean time for removal of external fixator, mean time to achieve union and mean time off work were significantly lower in the rhBMP-7 treated group compared to the control group [320]. BMP-7 has also been evaluated in a prospective randomized controlled trial for treatment of proximal pole scaphoid non-unions [321]. The patients were divided into three groups: Group 1 received autografts alone, Group 2 received autografts with rhBMP-7, and Group 3 received allograft with rhBMP-7. Clinical, radiological and scintigraphic assessment revealed that rhBMP-7 improved performance of both autografts and allografts. Radiological evidence of healing in patients treated with autografts and rhBMP-7 was four weeks in comparison to nine weeks for patients treated with autografts alone. Furthermore clinical outcome of patients treated with allograft and rhBMP-7 was equal to Group 1 where patients were treated with autografts alone [321]. In a prospective study, twenty-three consecutive patients with atrophic humeral diaphyseal non-unions were treated with compression plates or intramedullary nails in conjunction with rhBMP-7. The authors reported that union was achieved in all patients without any serious complications or adverse effects. They concluded that rhBMP-7 was safe and effective for treatment of humeral diaphyseal non-unions [322].

In a recent prospective single arm study [323], McKee and colleagues examined the effectiveness of rhBMP-7 for treatment of atrophic long bone non-unions. Participating patients on an average had 2.1 previous operations and autogenous iliac crest bone grafting had already failed in 28 of them. All the patients underwent revision fixation with application of rhBMP-7 at the non-union site. The authors reported that 54 of the original 61 non-union cases had healed at the conclusion of the study and that there were no anaphylactic reactions or adverse effects associated with the use of rhBMP-7 [323]. In another study, a case review was made of 14 patients who underwent treatment for lesions of the body and angle of the mandible resulting from neoplasms or osteomyelitis. The patients were treated with rhBMP-2 on a collagen carrier without concomitant use of bone materials. The study revealed that all the cases had successful osseous restoration of the edentulous area, which was subsequently followed by prosthetic treatment. The authors concluded that use of rhBMP-2 in treatment of critical sized mandibular defects without concomitant use of bone grafts resulted in excellent regeneration of affected area allowing the restoration of prosthodontic function [309].

5. Controversy with rhBMPs

Although BMPs are being studied extensively for bone tissue engineering and repair applications, controversy exists. This is regarding the success and failure of rhBMP-2 in conflicting reports. The muddled reporting of its clinical superiority to autografts from iliac crests and the failure to report or underreporting of adverse side effects from its use exist [15,16,324,325]. Since 2006, independent research studies started demonstrating 20%–70% failure rates with the use of rhBMP-2 [326]. Seroma formation, bone over growth, retrograde ejaculation and increased risk of neoplastic changes are the most common complications associated with their use. The FDA placed a warning on BMP use in June 1998 in cervical spine applications due to extreme postoperative dysphagia [325,327]. The *Wall Street Journal* reported that Medtronic was under investigation for off label use of INFUSE (rhBMP-2) [327]. Allegations came forward of cherry picked research results and fraud by the author in a study showing

the effective use of rhBMP-2 [328]. It was revealed that the author had a conflict of interest and financial ties to the manufacturer of rhBMP-2 [327]. This led to the reputation of rhBMP-2 being tarnished and its clinical usefulness questioned. The question that was being raised was that is the advantage gained by the use of BMP worth the risks it poses. In order to clear some of the confusion, systemic reviews and meta-analysis of results were conducted independently by Yale. The reports found that the current data in whole does not show a significant improvement in fusion rates with the use of rhBMP-2 as compared to autograft iliac crest bone graft used alone [325–327]. Both BMP-2 and iliac crest bone graft were shown to be associated with similar rates of neurological and retrograde ejaculation and complications when used in posterolateral or anterior interbody lumbar fusion [325,327]. It was concluded that BMP-2 use results in higher rates of ectopic bone formation in posterior lumbar interbody procedures and high rates of complication in anterior cervical procedures [326,327]. Although there is a slight risk of cancer with the use of BMP-2, the absolute risk remains extremely small and, therefore, clinically insignificant [18,327].

6. Future Challenges

The discovery of BMPs ushered a new era not only in understanding of bone physiology but also in development of new methods for treatment of defects that require orthopaedic and maxillofacial surgery [13,24]. During the last decade, many successful preclinical and clinical studies have been carried out which are a testament to the tremendous potential of BMPs for use in tissue engineering applications for treatment of bone defects. These cytokines have the unique capacity to initiate bone formation not only in osseous tissues but also in extra-skeletal sites [14,45]. It has taken more than 40 years of time and a great deal of painstaking research from the initial discovery of these cytokines by American Orthopaedic Surgeon Marshall Urist [14,26,329] to their approval for clinical use by FDA. A lot of work still needs to be done if more BMPs based tissue engineering constructs are to become available for routine clinical use. It would require elucidation of optimal therapeutic dosage, development of more efficient carriers and better understanding of local bone repair environment [14]. Further research will also be required to better define the variables such as route of administration and ideal scaffold material.

The use of BMPs based delivery systems is still in its early days, but recent clinical studies in humans suggest that a promising future will unravel in development of BMPs based products for orthopaedics, periodontics, maxillofacial surgery and other clinical situations. Up until this point most studies and clinical trials have focused on rhBMP-2 and rhBMP-7 but given that bone regeneration involves intricate interplay of network of molecules, it is likely that use of cocktail of molecules comprising of different BMPs may be a more suitable approach than a single molecule [13,28,29,330] and hence there is a great need for research to evaluate this potential approach. Similarly advances in the field of biomaterials will also increase the potential approaches for delivery of BMPs for treatment of bone defects. Novel strategies such as nanoparticles and injectable systems will allow restricted and site specific delivery of BMPs. Systems which could potentially deliver BMPs with angiogenic factors and cells could potentially enhance rate, volume and quality of newly formed bone [13,25].

Although there have been limited clinical trials in comparison to a large pool of preclinical studies for evaluation of BMPs for routine clinical application in humans but their results have demonstrated that BMPs are effective and there is evidence to suggest that in some situations their efficacy is

comparable to or even better than autografts [45]. Although controversy exists regarding their use due to side effects observed in spinal applications [327]. BMP-7 has been compared to autogenous bone graft harvested from iliac crest, currently considered the gold standard in bone grafting materials. Preclinical, clinical and long-term follow up studies have demonstrated safety and effectiveness of BMP-7 [25,331]. Furthermore, prospective clinical trials evaluating the effectiveness of rhBMP-2 and rhBMP-7 for treatment of bone fractures and non-unions have also shown very encouraging results. It is only pertinent that more research is done to expound the relative effectiveness of BMPs, interaction among different types of BMPs and characteristics of the cells responding to BMP signaling. Additionally, there is a great need to distinguish if there is a single pathway to efficient bone regeneration or different clinical scenarios that require more specific tissue engineering approaches. This would enable us to better understand the physiological process involved in bone healing allowing us to develop more efficient and effective tissue-engineered bone constructs.

The principles of inter-species BMP dose extrapolation are not completely understood currently and are applied with varying success in clinical scenarios. Simple scaling of drug doses used in preclinical experimental animal models to humans can be erroneous and misleading. The physicochemical properties of the BMPs used and/or the knowledge of interspecies differences in physiology can be used to improve drug dosing. However, differences in BMP transport via carrier scaffolds, the dose–response relationship and metabolism makes the assessment of accurate BMP dosing for clinical applications very difficult. The reported adverse effects of BMP clinical use give rise to several imperative questions that remain to be addressed. One challenge is that BMP therapeutics use microgram amounts while endogenous BMPs act within the nanogram level. Development of smarter biomaterial carrier for delivery of BMPs and other growth factors in a better-controlled fashion is required. The ever increasing applications for use of BMPs reaffirms that future of regenerative medicine, particularly of BMPs for bone tissue engineering is a bright one [13,18] and possibility of a tissue engineered bone construct as an alternate to autogenous bone graft may be a reality in not so distant future.

Author Contributions

Zeeshan Sheikh wrote the manuscript, performed literature search, compiled information to create the table on biomaterial carriers for BMP delivery, and was responsible for all corrections and revisions needed in the manuscript. Mohammad Ahmad Javaid wrote the manuscript performed literature search and compiled information to create the types of BMPs and their expression table. Nader Hamdan performed literature search, compiled the information to create the table on clinical studies using BMP and also contributed towards finalization of the manuscript. Raheel Hashmi performed literature search, wrote the sections which included problems and controversies with BMP use in clinical application and also contributed towards finalization of the manuscript.

Conflicts of Interest

There are no conflicts of interest for any of the authors who prepared this manuscript.

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