

TISSUE ENGINEERED BONE USING SELECT GROWTH FACTORS: A COMPREHENSIVE REVIEW OF ANIMAL STUDIES AND CLINICAL TRANSLATION STUDIES IN MAN

D. Gothard^{1,*}, E.L. Smith¹, J.M. Kanczler¹, H. Rashidi², O. Qutachi², J. Henstock³, M. Rotherham³, A. El Haj³,
K. Shakesheff² and R.O.C. Oreffo^{1,*}

¹Bone and Joint Research Group, University of Southampton, Southampton, UK

²Wolfson Centre for Stem Cells, University of Nottingham, Nottingham, UK

³Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent, UK

Abstract

There is a growing socio-economic need for effective strategies to repair damaged bone resulting from disease, trauma and surgical intervention. Bone tissue engineering has received substantial investment over the last few decades as a result. A multitude of studies have sought to examine the efficacy of multiple growth factors, delivery systems and biomaterials within *in vivo* animal models for the repair of critical-sized bone defects. Defect repair requires recapitulation of *in vivo* signalling cascades, including osteogenesis, chondrogenesis and angiogenesis, in an orchestrated spatiotemporal manner. Strategies to drive parallel, synergistic and consecutive signalling of factors including BMP-2, BMP-7/OP-1, FGF, PDGF, PTH, PTHrP, TGF- β 3, VEGF and Wnts have demonstrated improved bone healing within animal models. Enhanced bone repair has also been demonstrated in the clinic following European Medicines Agency and Food and Drug Administration approval of BMP-2, BMP-7/OP-1, PDGF, PTH and PTHrP. The current review assesses the *in vivo* and clinical data surrounding the application of growth factors for bone regeneration. This review has examined data published between 1965 and 2013. All bone tissue engineering studies investigating *in vivo* response of the growth factors listed above, or combinations thereof, utilising animal models or human trials were included. All studies were compiled from PubMed-NCBI using search terms including 'growth factor name', '*in vivo*', 'model/animal', 'human', and 'bone tissue engineering'. Focus is drawn to the *in vivo* success of osteoinductive growth factors incorporated within material implants both in animals and humans and identifies the unmet challenges within the skeletal regenerative area.

Keywords: Animal model, bone tissue engineering, BMP-2, BMP-7, clinical translation, FGF, human studies, *in vivo*, OP-1, PDGF, PTH, PTHrP, TGF- β 3, VEGF, Wnt proteins.

*Address for correspondence:

Dr David Gothard or Professor Richard O. C. Oreffo
Bone and Joint Research Group,
Centre for Human Development and Health,
University of Southampton, School of Medicine,
Institute of Developmental Sciences,
Mail Point 887, Southampton General Hospital,
Tremona Road, Southampton, SO16 6YD, UK

Telephone Number: 02381 208664 (DG)

or 02381 205260 (RO)

Fax Number: 02381 204221

E-mail: D.Gothard@soton.ac.uk
or richard.oreffo@soton.ac.uk

Note

Tables 1-5 are included within the text of the paper. However, **Supplementary Tables 1-9** can be accessed from a secondary supplementary document that is available from the eCM Journal webpage for this paper (<http://www.ecmjournal.org/journal/papers/vol028/vol028a13.php>)

Introduction

Tissue engineering utilises design and construction principles to manufacture replacement tissues exhibiting competent biological function (Tabata, 2003). Regeneration or repair of critical-sized bone defects by substitution of damaged or diseased tissues requires an ability to recapitulate developmental biology processes and control tissue morphogenesis. Manipulation of tissue development and morphogenesis can be achieved through delivery of inductive signals replicating native *in vivo* microenvironmental cues. Utilisation of select growth factors enables controlled cell differentiation towards specified lineages (Sundelacruz and Kaplan, 2009). Spatiotemporal orchestration of growth factors *in vivo* is critical to successful bone tissue engineering strategies (Reddi, 2000). The aim of this review was to assess the inductive signalling aspect of current bone tissue engineering strategies, and identify individual growth factors or combinations thereof which have shown *in vivo* success within animal models and have been scaled to large animals prior to clinical translation within humans. It is pertinent to understand current progress to evaluate optimum strategies that can be taken forward for further study.

There is currently a range of tissue-engineered solutions advocated for bone repair and yet there remains a need for demonstrable preclinical and clinical efficacy of materials with a proven capacity to repair bone damage resulting from disease, trauma or surgical intervention. It is estimated that 3.6 % of the UK population (of over 64 million) will suffer a bone fracture in their lifetime (Donaldson *et al.*, 2008). The risk of fracture increases with age and statistics show 1 in 3 women and 1 in 5 men over 50 years of age will experience an osteoporotic fracture; a growing concern in an increasingly aged population (van Staa *et al.*, 2001). The worldwide incidence of hip fracture is expected to increase 240 % and 310 % in women and men, respectively,

by 2050 (Baim and Leslie, 2012; Gullberg *et al.*, 1997), resulting potentially in a rise from 1.6 million cases per annum to between 4.5 and 6.3 million cases (Cooper *et al.*, 1992b). Osteoporosis is a major cause of hip fractures with equally significant financial tolls derived from both immediate medical treatment and post-treatment aid for reduced mobility, disability and increased dependency (Keene *et al.*, 1993; Leslie *et al.*, 2012). There are currently over 2 million osteoporosis sufferers in the UK alone whose medical treatment of related fractures (Borgstrom *et al.*, 2010a; Borgstrom *et al.*, 2010b; Strom *et al.*, 2013) is predicted to cost over £2 billion by 2020 (Burge *et al.*, 2001). Throughout the rest of Europe, osteoporosis related fractures are estimated to cost £51 billion by 2050 (Kanis and Johnell, 2005).

In addition, typically 10 % of fractures fail to repair resulting in non-union and increased socio-economic costs. There are two categories of non-union, hypertrophic (callus formation but without union) and atrophic (no callus formation). Many factors contribute to non-union fractures including avascular necrosis, bone apposition failure, poor or loss of fixation, infection, and soft tissue imposition within the defect site. A study in 2007 showed that humeral, tibial and femoral non-unions cost £15.5K, £16.3K and £17.2K, respectively, on a 'best-case scenario' (Kanakaris and Giannoudis, 2007). Non-union may also occur following spinal fusion surgery (posterolateral lumbar arthrodesis). Over 200,000 spinal fusion procedures are performed per annum in the US, yet non-union occurs in 10-40 % of patients undergoing single-level fusions (Boden, 2000). This is more frequent in patients undergoing multiple-level fusions. Increasing costs are expected in the future, as only a third of vertebral fractures come to clinical attention and are officially diagnosed (Cooper *et al.*, 1992a); it is estimated that up to 29 % of vertebral fractures may go unrecognised in Europe alone (Delmas *et al.*, 2005). Improved diagnosis of bone-related cancers is also expected to see rising costs for treatment. More than 2,000 cases are diagnosed per annum in the UK and more than 3,000 in the US.

It is thus important to understand the principles of the bone-healing cascade and manipulation with biomaterials and growth factors to aid development of successful tissue

engineering strategies for effective bone regeneration and repair (Berner *et al.*, 2012). This review examines data published between 1965 and 2013. All bone tissue engineering studies detailing an *in vivo* response of selected growth factors, including bone morphogenetic protein 2 (BMP-2), BMP-7/osteogenic protein 1 (OP-1), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), parathyroid hormone (PTH), parathyroid hormone-related protein (PTHrP), transforming growth factor beta 3 (TGF- β 3), vascular endothelial growth factor (VEGF) and Wnt proteins, or combinations thereof, utilising animal models or human trials were included. All studies were compiled from PubMed-NCBI using search terms including 'growth factor name', '*in vivo*', 'model/animal', 'human', and 'bone tissue engineering'. Focus is drawn to the *in vivo* success of osteoinductive growth factors both in animals and in humans.

Animal Model Selection

To understand and recapitulate the healing cascade, suitable bone defects must be established *in vivo* through the use of appropriate animal models. Animal models allow for standardisation or elimination of variables which contribute to the success or failure of tissue engineered materials; animals may be obtained from the same source or breed, and maintained under identical environmental conditions (Khan and Lane, 2004). The bone defects must not exhibit spontaneous healing during the lifetime of the animal (Horner *et al.*, 2010). These 'critical-sized' bone defects are dependent on multiple factors and remain difficult to define across anatomical location and species (Cooper *et al.*, 2010). Previous work has defined a critical-sized defect as "a segmental bone deficiency of a length that exceeds 2 to 2.5 times the diameter of the affected bone" (Gugala *et al.*, 2007). However, this definition is not often applied to defects within animal models, and the important parameters to report are defect size and location. Efficacy of any tissue-engineered constructs within these critical-sized defects is dependent on a number of variables detailed in Table 1 (Lindsey *et al.*, 2006; Reichert *et al.*, 2009; Rimondini *et al.*, 2005). Furthermore, when choosing a

Table 1. Factors affecting both study animal selection and efficacy of implanted constructs within these animal models.

Factors affecting construct efficacy <i>in vivo</i>	Factors affecting animal species selection
• Anatomic bone location	• Acquisition and treatment costs
• Animal species and age	• Animal breed and uniformity
• Animal state of health (disease states)	• Animal size relative to implant number and size
• Bone structure and complexity	• Availability
• Defect size (critical sized) and position (bone region)	• Biological characteristics analogous to humans
• Mechanical load and stress conditions	• Blood and biopsy sample size and number
• Mutational status (strain)	• Ethical considerations
• Nutrition	• Existing biological knowledge of the species
• Presence of adjacent soft tissues	• Handling and nature of the animal
• Presence of periosteum	• Normal activity level of the animal
• Post-surgical fixation	• Resistance to infection
• Time	• Study period and lifetime of the animal
• Vascularisation	• Tolerance to surgery and captivity

suitable species for study, additional factors also require further consideration as detailed in Table 1 (Pearce *et al.*, 2007; Reichert *et al.*, 2009). A review of the literature by O'Loughlin *et al.* (2008) demonstrated clear preferences towards particular species for fracture related studies, including rat (38 %), rabbit (19 %), mouse (15 %), sheep (11 %), dog (9 %) and goat (4 %). The remaining 4 % comprised a wide selection of less frequently investigated animal models. Currently, no single animal model provides a representative comparison for human bone repair; rather each animal model is selected to address a particular research question. Advantages and disadvantages of current large and small animal models as human comparisons for bone tissue research are shown in Table 2. Furthermore, it is apparent that, dependent on the research question, bones of the selected animal model should exhibit significant physiological and pathophysiological analogies to human bone, regarding both macro- and micro-structure (Table 3). If animal studies are to inform clinical translation then models should be carefully selected to best recapitulate the *in vivo* bone environment within humans. Table 3 details the structure of bone, highlighting parameters that require consideration prior to animal model selection (Egermann *et al.*, 2005; Liebschner, 2004). Some aspects of animal bone structure may be similar to that of humans; however, a balance should be struck with those that are different from humans. For example, preliminary investigations of bone biology and response to growth factor combinations could be assessed within small animals such as mouse or rat, as they provide a high-throughput *in vivo* model with similar biochemical composition to humans, and existing literature would help evaluate and interpret data. Systems could then be transferred to large animal models to assess candidate growth factors or combinations thereof in a functional setting analogous to humans. For example, defect regeneration strategies could be assessed within sheep long bone fractures as a model for large bone defects in humans, where the scale, mechanical loading and bone composition are similar to humans.

Growth Factor Delivery Vehicles

Following selection of an animal model and formation of a suitable critical-sized defect, a scaffold material exhibiting multifactorial properties is typically required to fill or bridge the defect site (Butler *et al.*, 2000). Orthopaedic materials currently employed in bone regeneration studies comprise organic bone substitutes, synthetic biomaterials and/or inorganic materials (Table 4). The suitability of a selected biomaterial scaffold is governed by four factors; *i*) biomimicry, *ii*) biocompatibility, *iii*) biodegradability and *iv*) biomechanics. Successful scaffolds are thought to be those that replicate host tissue 3D architecture (porosity and microstructure enabling cell migration and vascularisation) (Bonfield, 2006; Laschke *et al.*, 2008; Ma, 2008), and do not elicit an immunological or inflammatory response locally or systemically during either long or short-term integration. If degradation is required the material should degrade over time without production of toxic by-products, and endure mechanical and physiological stresses (Ghosh

and Ingber, 2007; Howard *et al.*, 2008; McMahon *et al.*, 2008; Semino, 2008). Hip replacement implants are often not biodegradable, rather these implant scaffolds are selected to exhibit corrosion resistance, durability and strength sufficient enough to last the lifetime of the patient (Schauss *et al.*, 2006). Biomechanical properties of interest include elasticity, thermostability and tensile strength of the constituent materials (El Haj *et al.*, 2005; Guan and Davies, 2004; Lendlein and Langer, 2002).

Many researchers believe that bone scaffold material should ideally replicate/incorporate the extracellular matrix (ECM) and thus influence cell attachment, migration, proliferation, differentiation and resultant bone tissue organisation (Green *et al.*, 2002; Karageorgiou and Kaplan, 2005; Shin *et al.*, 2003; Yang *et al.*, 2003). A variety of materials have been designed to address this challenge exhibiting either a bioactive osteoconductive surface (Takimoto *et al.*, 2003), enhanced functionality as a consequence of cell-scaffold surface topography interactions (Cohen *et al.*, 1993; Engel *et al.*, 2008), functionalisation with a bioactive coating, or impregnation with bioactive molecules (Murphy and Mooney, 1999; Zhang *et al.*, 2009a). Growth and development of functional engineered tissue is dependent on environmental cues, both physical and chemical (Burdick and Vunjak-Novakovic, 2009; Chan and Mooney, 2008; Quaglia, 2008). Implanted scaffolds can be designed as a delivery system for essential growth factors critical to cellular proliferation and osteogenic differentiation (Basmanav *et al.*, 2008; Cartmell, 2009; Kanczler *et al.*, 2008). Sustained release of encapsulated growth factors from implanted material scaffolds provides adequate localised osteoinduction at the defect site and has shown some success *in vivo* with respect to tissue engineered bone (Table 5) (Tabata, 2003).

Alternative vehicles have also been utilised to deliver selected growth factors *in vivo*. Rather than direct delivery, the gene(s) encoding the selected growth factor(s) can be introduced to the defect site by means of viral transduction or non-viral transfection. As seen in mouse studies administering BMP-2 as the choice growth factor, either viral (Gazit *et al.*, 1999) or plasmid vectors (Osawa *et al.*, 2009) can be directly delivered to the defect site (Dupont *et al.*, 2012), or pre-treated cells can be delivered (Kallai *et al.*, 2010).

Individual Growth Factors

Growth factor choice in a tissue engineering approach is critical for successful bone formation. Notable growth factors known to be important for bone regeneration include BMP-2, BMP-7/OP-1, FGF, PDGF, PTH, PTHrP, TGF- β 3, VEGF and Wnt proteins. These growth factors have been applied individually and in combination, through direct and indirect delivery vehicles (Table 5). *In vivo* paracrine and autocrine signalling cascades leading to bone formation are complex and rely on strict spatiotemporal interplay between select growth factors. Teasing apart the individual roles that each growth factor plays within bone development and healing systems is of the highest

Table 2. Advantages and disadvantages of large and small animal models for *in vivo* bone tissue engineering strategies and extrapolation for human clinical study.

Animal	Advantages	Disadvantages	Reference(s)
Large Models	Extrapolation to human studies		
Dog	<ul style="list-style-type: none"> • Tractable • Similar trabecular bone mineral density (BMD) • Similar biochemical composition • Considerable existing literature • Trained in recuperative regime 	<ul style="list-style-type: none"> • Breed variety • Ethical implications • High bone remodelling • High costs • High mechanical strength • High solid bone fusion • Low non-union • Quadrupedal gait 	(Aerssens <i>et al.</i> , 1998; Kimmel and Jee, 1982; Neyt <i>et al.</i> , 1998; Pearce <i>et al.</i> , 2007; Skurla and James, 2005)
Goat	<ul style="list-style-type: none"> • Large body size for multiple implants • Similar BMD and biochemical composition • Similar body weight • Similar bone remodelling rate • Tolerant of ambient conditions 	<ul style="list-style-type: none"> • Ethical implications • Fast revascularisation • High costs • Inquisitive nature • Quadrupedal gait 	(Lamerigts <i>et al.</i> , 2000; Leung <i>et al.</i> , 2001; Pearce <i>et al.</i> , 2007)
Pig	<ul style="list-style-type: none"> • Similar anatomy, biochemical composition, BMD, bone healing and bone morphology 	<ul style="list-style-type: none"> • Aggressive • Difficult to handle • Ethical implications • Excessive body weight • High costs • High growth rate • Quadrupedal gait 	(Aerssens <i>et al.</i> , 1998; Mosekilde <i>et al.</i> , 1993; Pearce <i>et al.</i> , 2007; Thorwarth <i>et al.</i> , 2005)
Pri-mates	<ul style="list-style-type: none"> • Phylogenetic proximity to humans • Similar skeletal structure • Similar BMD (dependent on sub-species) 	<ul style="list-style-type: none"> • Availability • Difficult to handle • Ethical implications • High costs 	(Khan and Lane, 2004)
Sheep	<ul style="list-style-type: none"> • Age mimics human ageing in bone and osteoid volume, and mineral apposition • Considerable existing literature • Docile • Similar body weight • Similar long bones structure • Similar biochemical and mineral composition 	<ul style="list-style-type: none"> • Age-dependent bone remodelling • Ethical implications • Haversian remodelling at 7-9years • High costs • High mechanical strength (adults) • High trabecular BMD • Quadrupedal gait 	(Aerssens <i>et al.</i> , 1998; Newman <i>et al.</i> , 1995; Pearce <i>et al.</i> , 2007; Ravaglioli <i>et al.</i> , 1996)
Small Models	Extrapolation to human studies		
Minipig	<ul style="list-style-type: none"> • Reduced growth rate • Reduced body mass • Similar anatomy, BMD, bone healing and bone morphology • Similar biochemical composition 	<ul style="list-style-type: none"> • Ethical implications • Limited clinical translation • Quadrupedal gait • Size limitation for implants 	(Aerssens <i>et al.</i> , 1998; Pearce <i>et al.</i> , 2007)
Mouse	<ul style="list-style-type: none"> • Availability • Considerable existing literature • Easy to handle • Enable disease state research • Feasibility studies prior to scale up • Immunodeficient - accept xenogenic material • Lifespan allows for age-related research 	<ul style="list-style-type: none"> • Ethical implications • High bone healing rate • Impractical bone fixation • Limited blood and biopsy samples • Limited clinical translation • Limited long term studies • Limited sampling • Quadrupedal gait • Size limitation for implants 	(Gomes and Fernandes, 2010; Liebschner, 2004; O'Loughlin <i>et al.</i> , 2008)
Rabbit	<ul style="list-style-type: none"> • Availability • Comparable long bone and lumbar structure • Considerable existing literature • Early skeletal maturity • Ease of handling and size • Feasibility studies prior to scale up 	<ul style="list-style-type: none"> • Different bone structure • Ethical implications • High bone turnover • Limited clinical translation • Quadrupedal gait • Size limitation for implants 	(Castaneda <i>et al.</i> , 2006; Liebschner, 2004; Pearce <i>et al.</i> , 2007)
Rat	<ul style="list-style-type: none"> • Availability • Considerable existing literature • Easy to handle • Enable disease state research • Feasibility studies prior to scale up • Immunodeficient - accept xenogenic material • Lifespan allows for age related research • Similar biochemical composition 	<ul style="list-style-type: none"> • Ethical implications • High bone remodelling • Limited blood and biopsy samples • Limited clinical translation • Limited long term studies • Quadrupedal gait • Size limitation for implants 	(Aerssens <i>et al.</i> , 1998; Gomes and Fernandes, 2010; Liebschner, 2004; O'Loughlin <i>et al.</i> , 2008)

Table 3. Factors affecting both study animal selection and efficacy of implanted constructs within these animal models.

Physiological and pathophysiological analogies to humans	Hierarchy of bone structure
<ul style="list-style-type: none"> Macro Structure 	<ul style="list-style-type: none"> Level 1 – Whole bone
Compact and cancellous bone	External and internal geometry
<ul style="list-style-type: none"> Micro Structure 	<ul style="list-style-type: none"> Level 2 – Architecture
Osteons Haversian bone Lamellae Trabeculae	Internal trabecular structure Haversian/interstitial structure Circumferential structure
<ul style="list-style-type: none"> Shape and curvature 	<ul style="list-style-type: none"> Level 3 – Tissue
Epiphysis Metaphysis Diaphysis	Individual trabeculae Individual osteons Cortical microbeam structure
<ul style="list-style-type: none"> Composition 	<ul style="list-style-type: none"> Level 4 – Lamellar
Bone mineral content and density Collagen, proteoglycans and glycoproteins	Individual lamellae structure
<ul style="list-style-type: none"> Healing and Remodelling 	<ul style="list-style-type: none"> Level 5 – Ultrastructure
Callus formation Osteoclast resorption Osteoblast activity	Molecular composition Mineral composition

Table 4. Organic, inorganic and synthetic materials for orthopaedic applications *in vivo*.

Organic Substitutes for Bone		Synthetic and Inorganic Substitutes	
Name	Abbrev.	Name	Abbrev.
Alginate	ALG	Calcium carbonate	CaCO ₃
Allograft	<i>n/a</i>	Calcium deficient HA	CDHA
Autograft	<i>n/a</i>	Calcium phosphate	<i>n/a</i>
Chitosan	<i>n/a</i>	Carboxymethyl cellulose	CMC
Collagen	<i>n/a</i>	Cholesterol-bearing pullulan nanogel with acryloyl residue	CHPA
Coral	<i>n/a</i>	Hydroxyapatite	HA
Cortico-cancellous human bone block	CHBB	Poly ethylene glycol	PEG
Demineralised bone	<i>n/a</i>	Poly ethylene glycol-diacrylate	<i>n/a</i>
Deproteinised bovine bone block with porcine collagen	DBBB	Poly lactic co-glycolic acid	PLGA
Fibrin	<i>n/a</i>	Perfluorotributylamine	PFTBA
Fibrinogen	<i>n/a</i>	Poly L lysine	PLL
Gelatin	<i>n/a</i>	Polystyrene	<i>n/a</i>
Hyaluronic acid	HAA	Poly urethane	PUR
Matrigel	<i>n/a</i>	Poly vinyl alcohol	PVA
Monoolein	<i>n/a</i>	Poly glycolic acid	PGA
Silk fibroin	<i>n/a</i>	Poly caprolactone	PCL
		Poly propylene fumarate	PPF
		Poly lactic acid	PLA
		Poly-(N-isopropylacrylamide-co-acrylic acid)	pNIPAm-co-AAc
		Silica	<i>n/a</i>
		Titanium mesh	<i>n/a</i>
		Tri-calcium phosphate	TCP

importance to any robust and effective tissue engineering strategy. Here, the authors discuss the effect of growth factor delivery on bone formation *in vivo* following ‘direct’, ‘indirect’ and ‘combination’ administration. Within each section, the effect of administration within first of all ‘small’ animal models is discussed, followed by the effect within ‘large’ animal models. The incorporation of cells within tissue engineering strategies and their effect on bone formation is discussed case by case throughout the review.

BMP-2

The discovery of auto-induced bone formation in rabbits implanted with autologous demineralised, lyophilised bone segments by Marshall R. Urist in 1965 (Urist, 1965) led to the identification of osteoinductive signalling molecules named by Urist as ‘bone morphogenetic proteins’ (BMPs) (Urist and Strates, 1971). BMPs act as morphogens providing crucial signals which direct cell differentiation and tissue architecture. To date, twenty human BMP proteins have been discovered, of which eight (BMP-1 to

Table 5. *In vivo* bone tissue engineering utilising growth factors including BMP-2, BMP-7, FGF, PDGF, PTH, PTHrP, TGF- β 3, VEGF and Wnt proteins. (divided into 4 parts - part 1)

Growth Factor(s)	Animal Model	Defect location and type	Time	Delivery system	Dose/ Conc.	Defect regeneration	Analysis methods
1. Direct Delivery							
BMP-2	Large Models	Dog, Goat, Horse, Monkey, Pig and Sheep	3 weeks to 26 months	<ul style="list-style-type: none"> Organic scaffolds – collagen, demineralised bone, gelatin and silk fibroin Inorganic scaffolds – CPC, ceramic phosphate, HA, PCL, PEG, PLA, PLGA, TCP and titanium oxide 	5 to 100,000 μg 1.6 to 1,500 $\mu\text{g}/\text{mL}$	1.2 to 21 fold (bone) 1.7 to 2.6 fold (biomechanics)	Biochemistry, biomechanical testing, CT (micro), DXA, faxitron, histology, histomorphometry, immunohistochemistry, <i>in situ</i> hybridization, MRI, RT-PCR, radiography (micro) and SEM
	#37						
	Small Models						
	#62	<ul style="list-style-type: none"> Drill – calvaria, cleft, condyle, patella and vertebrae Ectopic – intramuscular and subcutaneous Fusion – lumbar Segmental – femur, fibula, humerus, radius, tibia, ulna (endochondral), mandible, maxilla, periodontal ridge and zygomatic arch (intramembranous) 	5 d to 24 weeks	<ul style="list-style-type: none"> Organic scaffolds – alginate, CHBB, chitosan, collagen, coral-line HA, DBBB, demineralised bone, fibrin, gelatin, hyaluronan and monolein Inorganic scaffolds – CDHA, CHPA, CPC, PCL, PEG, PLA, PLG, PLGA, polystyrene, PUR, PVA, silica and TCP 	0.1 to 5,000 μg (other studies have used 150,000 μg) 2 to 4,000 $\mu\text{g}/\text{mL}$	1.1 to 50 fold (bone) 1.6 to 18 fold (biomechanics)	
BMP-7/ OP-1	Large Models	Baboon, Dog, Goat, Monkey and Sheep	1 week to 1 year		100 to 750,000 μg 1,000 to 3,500 $\mu\text{g}/\text{mL}$	1.25 to 8.3 fold (bone) 1.65 to 3.3 fold (biomechanics) 1.6 fold (osteoid)	Biomechanical testing, CT (micro), DXA, histology, histomorphometry, immunohistochemistry, MRI, radiography (micro), radioimmunoassay, and SEM
	#28						
	Small Models						
	#25	<ul style="list-style-type: none"> Drill – calvaria, femur, humerus and condyle Ectopic – intramuscular and subcutaneous Fusion – lumbar Osteotomy – tibia and mandible OVX – osteopenia Segmental – femur, tibia, ulna (endochondral), alveolar ridge and mandible (intramembranous) 	5 d to 12 weeks	<ul style="list-style-type: none"> Organic scaffolds – alginate, allograft, autograft, chitosan, collagen, DBM and xenograft Inorganic scaffolds – CMC, HA, hydroxylapatite, PCL, PLGA, PLLA, polylactide, TCP and titanium 	0.025 to 3,500 μg 25 to 200 $\mu\text{g}/\text{mL}$	1.1 to 29.5 fold (bone) – one study showed 96 fold 1.3 to 31 fold (biomechanics)	
FGF-1/2/18	Large Models	Dog and Primate	2 to 32 weeks		0.15 to 200 μg 100 to 400 $\mu\text{g}/\text{mL}$	1.3 to 3 fold (bone)	Biochemistry, biomechanical testing, CT (micro), FTIR, histology, histomorphometry, immunohistochemistry, radiography and SEM
	#5						
	Small Models	Mouse, Rabbit and Rat	3 d to 24 weeks	<ul style="list-style-type: none"> Direct injection Organic scaffolds – collagen, gelatin and Matrigel Inorganic scaffolds – HA, hydroxypropyl cellulose, polyethylene, polyglycolate, polylactide, TCP and titanium 	0.01 to 200 μg 10 to 100 $\mu\text{g}/\text{mL}$ 100 to 1,000 $\mu\text{g}/\text{kg}$	1.2 to 16.4 fold (bone) 2.1 to 4 fold (biomechanics)	
	#20						
PDGF	Small Models	Minipig, Mouse, Rabbit and Rat	10 d to 12 weeks	<ul style="list-style-type: none"> Organic scaffolds – chitosan, collagen, DBM and fibrin Inorganic scaffolds – HA, PDLLA, PGA, PLGA, PLLA and TCP 	0.01 to 750 μg 1 to 1,000 $\mu\text{g}/\text{mL}$	1.45 to 10 fold (bone)	Biochemistry, biomechanical testing, histology, histomorphometry, immunohistochemistry, micro CT, radiography and SEM
	#14						
PTH (1-31, 1-34, 1-84, 2-34, 28-48 and 53-84)	Large Models	Dog, Monkey and Sheep	4 weeks to 4.5 years		0.75 to 7.5 $\mu\text{g}/\text{kg}/\text{d}$ 20 to 1,000 $\mu\text{g}/\text{mL}$	1.1 to 3.4 fold (bone) 1.4 to 2 fold (mechanics)	Biochemistry, biomechanical testing, DXA, finite element analysis, histology, histomorphometry, <i>in situ</i> hybridisation, immunohistochemistry, manual palpation, micro CT, northern blot, QCT, radiography, radiolabelling, RT-PCR and SEM.
	#6						
	Small Models	Mouse, Rabbit and Rat	1 h to 1 d, 2 d to 24 months	<ul style="list-style-type: none"> Subcutaneous injection Organic scaffolds – fibrin and RGD Inorganic scaffolds – calcium phosphate, HA, PEG and TCP 	0.05 to 800 $\mu\text{g}/\text{kg}/\text{d}$ 20 to 100 $\mu\text{g}/\text{mL}$	1.1 to 13.1 fold (bone) 1.1 to 3.8 fold (mechanics)	
	#46						
PTHrP peptides (1-36 and 107-139)	Small Models	Mouse, Rabbit and Rat	12 d to 6 months	<ul style="list-style-type: none"> Subcutaneous injection Inorganic scaffold – silica 	10 to 320 $\mu\text{g}/\text{kg}/\text{d}$	1.2 to 10 fold (bone)	Biomechanical testing, CT (micro), histology, histomorphometry, immunohistochemistry, RT-PCR and western blot
	#9						
TGF-β3	Large Model	Baboon	30 to 90 d	Organic scaffolds – collagen and Matrigel	75 to 125 μg	1.6 to 3 fold (bone)	Biomechanical testing, CT (micro), histology, histomorphometry, immunohistochemistry, RT-PCR and western blot
	#3						
	Small Models	Mouse and Rat	14 to 28 d	Organic scaffolds – calcium phosphate and collagen	0.003 to 2.75 μg	1.05 fold (bone) 1.2 fold (cartilage)	
	#2						
VEGF	Small Model	Mouse	4 weeks	<ul style="list-style-type: none"> Organic scaffolds – calcium phosphate Inorganic scaffolds – PLA 	1.7 μg 5 $\mu\text{g}/\text{mL}$	1.65 fold (bone) Enhanced vascularisation	CT (micro), histology, histomorphometry, immunohistochemistry, intravital microscopy and radiography
	#2						

Table 5. *In vivo* bone tissue engineering utilising growth factors including BMP-2, BMP-7, FGF, PDGF, PTH, PTHrP, TGF- β 3, VEGF and Wnt proteins. (continued - part2)

Growth Factor(s)	Animal Model		Defect location and type	Time	Delivery system	Dose/ Conc.	Defect regeneration	Analysis methods
1. Direct Delivery (continued)								
Wnt 3A	Small Model	Mouse	<ul style="list-style-type: none">Delayed skeletal development – suture closureSegmental – tibia (endochondral)	4 weeks	<ul style="list-style-type: none">Injection – protein suspension and liposomal vesicles	0.5 µg/mL	Enhanced bone healing and reduced suture area approx. 1.4 fold	Biochemical testing, CT (micro), histology, immunohistochemistry, <i>in situ</i> hybridisation and RT-PCR
	#2							
2. Indirect Delivery								
BMP-2	Large Models	Dog, Horse, Pig and Sheep	<ul style="list-style-type: none">Drill – calvaria, iliac crest, orbital bone (lacrimal) and patellaEctopic – intra-muscular and subcutaneousFusion – lumbarSegmental – femur, fibula, metacarpal, metatarsal, radius (endochondral) and mandible, maxilla (intramembranous)	4 to 24 weeks	<ul style="list-style-type: none">Direct viral/non-viral particle injection or implant of transduced/transfected cells with and without scaffoldsViral transduction – adenovirus, retrovirus, lentivirusNon-viral transfection – plasmids and vectorsOrganic scaffolds – alginate, allograft, autograft, collagen, demineralised bone matrix, fibrin, fibrinogen and MatrigelInorganic scaffolds – PEG-diacrylate, PFTBA and titanium mesh	12 µg 0.04 to 5 x 10 ¹¹ viral particles 2 to 5 x 10 ⁷ cells	1.3 to 3.2 fold (bone)	Biomechanical testing, CT (micro), DXA, histology, histomorphometry, immunohistochemistry, <i>in vivo</i> imaging, RT-PCR, and radiography (micro)
	#6			1 to 35 weeks		3 to 75,000 µg 0.001 to 7 x 10 ¹⁰ viral particles 0.005 to 5 x 10 ⁷ cells	1.3 to 9 fold (bone) 2.7 to 10.9 fold (biomechanics)	
	Small Models	Minipig, Mouse, Rabbit and Rat						
	#32							
BMP-7/OP-1	Large Models	Dog and Goat	<ul style="list-style-type: none">Ectopic – intramuscularIntervertebral disc transplantSegmental – femur (endochondral) and mandible (intramembranous)	1 to 8 months	<ul style="list-style-type: none">Direct viral/non-viral particle injection or implant of transduced/transfected cells with and without scaffoldsViral transduction – adenovirusNon-viral transfection – plasmids and vectorsOrganic scaffolds – allograft, chitosan, collagen, coral, gelatin and silk fibroinInorganic scaffolds – HA, PA and PCL	2 x 10 ¹⁰ viral particles 0.01 to 5 x 10 ⁷ cells	2 to 2.5 fold (bone)	Biomechanical testing, biochemistry, CT(micro), cytochemistry, histology, histomorphometry, MRI, radiography, RT-PCR and SEM
	#3			1 to 16 weeks		25 to 250 µg 0.2 to 2.5 x 10 ¹¹ viral particles 0.1 to 4 x 10 ⁶ cells	1.03 to 5 fold (bone) – one study showed 21 fold	
	Small Models	Mouse, Rabbit and Rat						
	#11							
FGF-2	Large Models	Dog	<ul style="list-style-type: none">Furcation – dental rootIrradiationDrill – calvariaSegmental – radius (endochondral)	6 weeks	<ul style="list-style-type: none">Direct implantation of transduced cells within scaffoldInorganic scaffolds – HA, PA66, PLGA and TCP	unknown	Enhanced periodontal bone regeneration	Biochemistry, clinical examination, CT (micro), DXA, histology, histomorphometry, immunohistochemistry, pQCT, radiography, RT-PCR and SEM
	#1			1 to 20 weeks		0.0625 to 5 x 10 ⁶ cells	2 to 2.7 fold (bone) – one study showed 53.5 fold	
	Small Models	Mouse, Rabbit and Rat						
#5								
PDGF	Small Models	Mouse and Rat	<ul style="list-style-type: none">Ectopic – subcutaneousSegmental – alveolar ridge and femur	10 d to 6 weeks	<ul style="list-style-type: none">Direct viral/non-viral particle injection or implant of transduced/transfected cells with and without scaffoldsViral transduction – adenovirusNon-viral transfection – plasmids and vectorsOrganic scaffolds – collagen, methylcellulose and silkInorganic scaffolds – PLGA and mesoporous glass	5.5 x 10 ⁶ to 5.5 x 10 ⁹ PFU/mL 1 x 10 ⁶ cells	1.7 to 2 fold (bone)	Backscatter SEM, biochemistry, biomechanical testing, histology, histomorphometry, immunohistochemistry, micro CT, northern blot and RT-PCR
	#4							
TGF-β3	Small Model	Mouse	<ul style="list-style-type: none">Ectopic - subcutaneous	30 d	<ul style="list-style-type: none">Direct implantation of transduced cells within scaffoldViral transduction – recombinant adeno-associated virusInorganic scaffold – PLLA/PEG scaffold	1 x 10 ⁶ cells	3D cartilage constructs	Histology, immunohistochemistry and western blot
	#1							
VEGF	Small Models	Mouse and Rabbit	<ul style="list-style-type: none">Ectopic – subcutaneousSegmental – femur, radius and tibia (endochondral)	4 to 16 weeks	<ul style="list-style-type: none">Direct delivery of non-viral particles or implantation of transduced cellsNon-viral transfection – plasmid vectorsOrganic scaffolds – collagen, calcium phosphate and calcium carbonate	20 µg 5 x 10 ⁶ cells	1.6 to 2 fold (bone) Enhanced vascularisation	CT (micro), histology, histomorphometry, immunohistochemistry, RT-PCR and radiography
	#3							
Wnt 1, 3A, 4, 5A, 6 and 10B	Small Models	Chick, Mouse and Rat	<ul style="list-style-type: none">Drill – calvariaSCID mouseTransfected embryo	4 d to 12 weeks	<ul style="list-style-type: none">Direct implantation of transduced/transfected cells with and without scaffoldsViral transduction – lentivirus and retrovirusNon-viral transfection – plasmid vectorsOrganic scaffold – HA and TCPInorganic scaffold – PLGA	0.15 to 5 x 10 ⁶ cells N/A (transgenic animal)	1.25 to 12 fold (bone) 1.5 fold (cartilage)	CT (micro), histology, histomorphometry, immunohistochemistry, <i>in situ</i> hybridisation and radiography
	#8						Wnt-5A reduced bone formation	
3. Combinational Delivery								
BMP-2 <i>plus</i> (BBP, BMP-7/OP-1, Epo, FGF, integrin, MSCs, Runx2, TGF-β2, tobramycin, VEGF or zoledronic acid)	Large Model	Dog, Horse and Pig	<ul style="list-style-type: none">Drill – calvaria, orbital bone (lacrimal), and ulnaEctopic – intramuscular and subcutaneousFusion – lumbarIrradiated – mandibleSegmental – femur and tibia (endochondral)	1 to 9 weeks	<ul style="list-style-type: none">Direct delivery by injection or implant, and indirect delivery by viral/non-viral particle injection or transduced/ transfected cell implantOrganic scaffolds – alginate, allograft, chitosan, collagen, coral and gelatinInorganic scaffolds – CMC, CDHA, PEG-diacrylate, PEG-MMP, PLA, PLGA, PPF, TCP and titaniumViral transduction – adenovirus, baculovirus and lentivirusNon-viral transfection – plasmids	5.26 to 120 µg BMP-2 2 x 10 ¹¹ viral particles	1.6 fold (bone)	Biochemistry, biomechanical testing, CT (micro), DXA, histology, histomorphometry, immunohistochemistry, <i>in situ</i> hybridisation, RT-PCR, PET (micro), radiography, rheology and western blot
	#4			1 to 16 weeks		0.0025 to 200 µg BMP-2 0.075 to 7.5 x 10 ¹⁰ viral particles 1 to 4.8 x 10 ⁶ cells	1.1 to 20 fold (bone) 4 to 8.3 fold (biomechanics) 2 fold (vasculature)	
	Small Models	Minipig, Mouse, Rabbit and Rat						
	#38							

Table 5. *In vivo* bone tissue engineering utilising growth factors including BMP-2, BMP-7, FGF, PDGF, PTH, PTHrP, TGF- β 3, VEGF and Wnt proteins. (continued - part 3)

Growth Factor(s)	Animal Model		Defect location and type	Time	Delivery system	Dose/ Conc.	Defect regeneration	Analysis methods
3. Combinational Delivery (continued)								
BMP-7/OP-1 <i>plus</i> (BBP, BMP-2, BMSCs, blood, bone marrow, FGF-2, IGF-1, pamidronate, PDGF β , PTH (1-34), MSCs, osteoblasts, TGF- β 1, TGF- β 3, TSP-1 or VEGF)	Large Models	Baboon, Dog and Horse	<ul style="list-style-type: none">• Drill – calvaria and femur• Ectopic – intramuscular and subcutaneous• Fusion – lumbar• Osteotomy – tibia• OVX – osteopenia• Segmental – mandible and metacarpal (endochondral and intramembranous)	2 to 16 weeks	Direct delivery by injection or implant, and indirect delivery by viral/non-viral particle injection or transduced/ transfected cell implant <ul style="list-style-type: none">• Organic scaffolds – allograft, chitosan, collagen, gelatin and silk fibroin• Inorganic scaffolds – calcium carbonate, calcium phosphate, CMC, HA, PCL, PDLLA and TCP• Viral transduction – adenovirus• Non-viral transfection – plasmids	5 to 5,000 μg – one study used 125mg 0.2 to 2 x 10 ¹¹ viral particles	1.4 to 5.3 fold (bone) 3 fold (osteoid)	Biochemistry, biomechanical testing, CT (micro), cytochemistry, DXA, histology, histomorphometry, immunohistochemistry, MRI, radiography, RT-PCR and SEM
	#7							
	Small Models	Mouse, Rabbit and Rat		1 d to 48 weeks		2 to 200 μg 0.00075 to 5.5 x 10 ¹¹ viral particles 1 to 4 x 10 ⁶ cells	1.2 to 15 fold (bone) 1.2 to 1.4 fold (biomechanics)	
	#19							
FGF-2 <i>plus</i> (17 β -estradiol, BMP-7/OP-1, estrogen, IGF-2, PTH (1-34), melatonin or VEGF)	Small Models	Mouse, Rabbit and Rat	<ul style="list-style-type: none">• Drill – calvaria, condyle and femur• Ectopic – intramuscular• OVX – osteopenia	1 to 48 weeks	Direct infusion or scaffold implant <ul style="list-style-type: none">• Organic scaffolds – collagen and gelatin• Inorganic scaffolds – titanium	0.001 to 100 μg 200 to 1,000 $\mu\text{g/mL}$	1.1 to 3.3 fold (bone) 8 fold (osteoid)	Biochemistry, CT (micro), DXA, histology, histomorphometry, immunohistochemistry, radiography and XTM
	#11							
PDGF <i>plus</i> (bFGF, BMP-2, BMP-7, BMSCs,IGF-1, osteogenin, TGF- β 1 and VEGF)	Large Models	Dog	<ul style="list-style-type: none">• Drill – calvaria• Ectopic – subcutaneous• Osteotomy – mandible• Segmental – femur	4 to 18 weeks	Direct delivery by implant, and indirect delivery by viral particle injection <ul style="list-style-type: none">• Organic scaffolds – chitosan, collagen, fibrinogen and methylcellulose• Inorganic scaffolds – Brushite, calcium phosphate, ePTFE, TCP and titanium• Viral transduction – adenovirus	5 $\mu\text{g/mL}$ 2 x 10 ¹⁰ viral particles	1.4 to 2.3 fold (bone)	Flow cytometry, histology, histomorphometry and micro CT
	#4							
	Small Models	Mouse, Rabbit and Rat		4 to 8 weeks		0.05 to 200 μg 0.001 to 0.05 $\mu\text{g/mL}$	2.5 to 10 fold (bone)	
	#6							
PTH (1-34 and 1-84) <i>plus</i> (alendronate, BMP-2, BMP-7, BMSCs, human PDL cells, ibandronate, IL-6, MSCs, pamidronate, rapamycin, tiludronate and zoledronic acid)	Large Models	Sheep	<ul style="list-style-type: none">• Ectopic – subcutaneous• Fracture – tibia• OVX – osteopenia• Segmental - femur and tibia	3 months	<ul style="list-style-type: none">• Subcutaneous injection	500IU/day	2 to 4 fold (bone) PTH alone	Biochemistry, mechanical testing, DXA, FACS, faxitron, histology, histomorphometry, immunocytochemistry, immunohistochemistry, micro CT, nanoindentation testing, northern blot, QCT, radiography, raman spectroscopy, RT-PCR, SEM and western blot
	#1							
	Small Models	Mouse, Rabbit and Rat		1 to 15 weeks		10 to 90 $\mu\text{g/kg/d}$	1.2 to 4.1 fold (bone) 1.2 to 3.1 fold (mechanics)	
	#14							
PTHrP peptides (1-36 and 1-86) <i>plus</i> (C-terminal PTHrP (107-139) peptide or PTH)	Small Model	Mouse	<ul style="list-style-type: none">• Diabetes• Knockout – PTHrP• Osteopenia	1 to 2 months	<ul style="list-style-type: none">• Subcutaneous injection	80 to 100 $\mu\text{g/kg/d}$	1.5 to 3 fold (bone)	Biochemistry, CT (micro), DXA, faxitron analysis, histology, immunohistochemistry, RT-PCR, radiography and western blot
	#3							
TGF-β3 <i>plus</i> (BMP-2, chondrocytes, MSCs, OP-1, Sox9 or TGF- β 1)	Large Models	Baboon and Sheep	<ul style="list-style-type: none">• Drill – condyle, humerus and patella• Ectopic – subcutaneous and intra-muscular	63 to 90 d	<ul style="list-style-type: none">• Organic scaffold – chitosan and fibrin• Inorganic scaffold – HA and calcium carbonate	0.05 to 125 μg TGF- β 3	5.3 fold (bone)	Biomechanical testing, CT (micro), histology, histomorphometry, immunohistochemistry and RT-PCR
	#2							
	Small Models	Mouse, Rabbit and Rat		1 to 22 weeks		Direct infusion or scaffold implant <ul style="list-style-type: none">• Organic scaffolds – alginate and fibrin• Inorganic scaffolds – PEG-PCL, PLGA, PLL and pNIPAm-co-AAc	0.02 μg TGF- β 3 (0.01 to 0.1 $\mu\text{g/mL}$)	
VEGF <i>plus</i> (BMP-2, BMP-4, BMP-7/OP-1 or FGF-2)	Large Model	Dog and Pig	<ul style="list-style-type: none">• Drill – calvaria• Ectopic – subcutaneous and intramuscular• Segmental – femur, ulna (endochondral) and infra-orbital bone (intramembranous)• Sinus floor elevation	9 weeks	Direct delivery by injection or implant, and indirect delivery by viral/non-viral particle injection or transduced/ transfected cell implant <ul style="list-style-type: none">• Organic scaffolds – alginate, bioacoral, collagen, gelatin and silk hydrogel• Inorganic scaffolds – calcium phosphate, PLA, PLGA, PPF, octacalcium phosphate and TCP• Viral transduction – adenovirus and retrovirus• Non-viral transfection – plasmids and vectors	0.4 to 4 μg	1.6 fold (bone) Enhanced vascularisation	Biochemistry, CT (micro), histology, histomorphometry, immunohistochemistry, <i>in situ</i> hybridisation, microangiography, radiography and SEM
	#2							
	Small Models	Mouse, Rabbit and Rat		1 to 16 weeks		0.2 to 20 μg 5.5 x 10 ¹¹ viral particles 0.2 to 3 x 10 ⁶ cells	1.4 to 20 fold (bone) 4 to 208 fold (biomechanics) 2 fold (vasculature)	
	#17							
4. Human Trials								
BMP-2	Human		<ul style="list-style-type: none">• Bone augmentation• Facial reconstruction (cleft, mandible and maxilla)• Long bone fracture and non-union (tibia)• Lumbar fusion	6 weeks to 6 years	<ul style="list-style-type: none">• Organic scaffolds – allograft, autograft, collagen and gelatin• Inorganic scaffolds – fusion cage, HA-TCP, PEEK, PGA, PLGA, PLA and titanium mesh	0.9 to 100 mg (0.75 to 1.5 mg/mL)	Enhanced bone healing was observed in the majority of patients	Biochemistry, CT, histology and radiography
	#30							

Table 5. *In vivo* bone tissue engineering utilising growth factors including BMP-2, BMP-7, FGF, PDGF, PTH, PTHrP, TGF- β 3, VEGF and Wnt proteins. (continued - part 4)

Growth Factor(s)	Animal Model	Defect location and type	Time	Delivery system	Dose/ Conc.	Defect regeneration	Analysis methods
4. Human Trials (continued)							
BMP-7/ OP-1	Human	<ul style="list-style-type: none"> Mandible reconstruction Non-union fracture Long bone osteotomy Lumbar fusion Pseudarthrosis 	2 weeks to 68 months	<ul style="list-style-type: none"> Organic scaffolds – allograft, autograft, collagen and xenograft Inorganic scaffolds – CMC, TCP and titanium 	2.5 to 17.5 mg – one study used 2,000 mg (3.5mg average)	Accelerated bone healing and increased bone tissue and mechanical strength was observed	Clinical assessment, CT, histology, histomorphometry, physical examination, radiography and scintigraphy
	#32						
PDGF	Human	<ul style="list-style-type: none"> Periodontitis 	9 months	<ul style="list-style-type: none"> Organic scaffolds – allograft 	0.5 to 5 mg/mL	1.12 to 1.17 fold (bone)	Clinical assessment, histology and radiography
	#1						
PTH	Human	<ul style="list-style-type: none"> Healthy adults Low bone mineral density Mandibular repair Postmenopausal women Vertebral fracture 	2 months to 2 years (one study - 7 d)	<ul style="list-style-type: none"> Subcutaneous injection 	20 to 100 μ g/d 2 to 4 pmol/kg/h	Accelerated bone healing through upregulation of bone markers and resultant bone tissue was observed	Biochemistry, clinical assessment, CT, DXA, histology, histomorphometry, QCT, quality of life assessment, radiography, SEM and TEM
	#14						
PTHrP peptides (1-34 and 1-36)	Human	<ul style="list-style-type: none"> Healthy adults Postmenopausal estrogen deficient females 	2 d to 2 weeks	<ul style="list-style-type: none"> Subcutaneous injection Intravenous infusion 	2 to 80 pmol/kg/h	Bone formation was activated in postmenopausal females, but inhibited in healthy adults	Biochemistry
	#4						

Abbreviations: 1,24,25[OH]₂D₃ (1,24,25-trihydroxyvitamin D₃), 2MD (2-methylene-19-nor-(20S)-calcitriol), BBP (BMP binding protein), BMP-2/7 (bone morphogenetic protein 2/7), CDHA (calcium deficient hydroxyapatite), CHBB (cortico-cancellous human bone block), CHPA (cholesterol-bearing pullulan nanogel with acryloyl residue), CMC (carboxymethylcellulose), CPC (calcium phosphate cement), CT (computerised tomography), DBBB (deproteinised bovine bone block/porcine collagen), DBM (demineralised bone matrix), DXA (dual energy x-ray absorptiometry), Epo (erythropoietin), FGF (fibroblast growth factor), FTIR (fourier transform infrared spectroscopy), HA (hydroxyapatite), HAA-PVAH (hyaluronic acid and poly-vinyl alcohol functionalised with hydrazide groups), ID₂ (1 α -hydroxyvitamin D₂), ID₃ (1 α -hydroxyvitamin D₃), MMP (matrix metalloproteinase), MRI (magnetic resonance imaging), MSC (mesenchymal stem cell), OP-1 (osteogenic protein-1), ORX (orchiectomised), OVX (ovariectomised), PCL (poly caprolactone), PDLLA (poly-D, L-lactide acid), PEEK (polyetheretherketone), PEG (poly ethylene glycol), PET (positron emission tomography), PGA (poly glycolic acid), PLA (poly lactic acid), PLG (poly D,L-lactide-co-glycolide), PLGA (poly lactic co-glycolic acid), PLL (poly-L-lysine), PLLA (poly-L-lactide), pNIPAm-co-AAc (poly-(N-isopropylacrylamide-co-acrylic acid)), PPF (poly-propylene fumarate), pQCT (peripheral quantitative CT), PTfBA (perfluorotributylamine), PTFE (polytetrafluoroethylene), PTH (parathyroid hormone), PTHrP (parathyroid hormone receptor-related protein), PUR (polyurethane), PVA (poly-vinyl alcohol), PX (sex determining region Y)-box 9), TCP (tricalcium phosphate), TEM (transmission electron microscopy), TGF β (transforming growth factor β), VEGF (vascular endothelial growth factor), VitD₃ (vitamin D₃), Wnt (wingless-type MMTV integration site family), and XTM (X-ray tomographic microscopy). # - number of publications.

BMP-8a) have a known osteochondral function (Even *et al.*, 2012). BMP-2 specifically is a disulfide-linked homodimer with a known role in osteoblast differentiation. Abundant use of recombinant human BMP-2 (rhBMP-2) within animal models has demonstrated successful *in vivo* bone regeneration and repair, and has been extensively examined as an osteoinductive growth factor for tissue engineering (Supplementary Table 1).

Direct administration of BMP-2

A review of the literature revealed variable increased bone formation and defect regeneration ranging between 1.2 and 21 fold in large animal models (He *et al.*, 2009; Wikesjo *et al.*, 2008), and 1.1 and 50 fold in small animal models (Ishihara *et al.*, 2008; Tolli *et al.*, 2011) (Table 5). To date, despite the wealth of reported studies there remains a lack of consensus concerning the optimum rhBMP-2 dose for effective bone defect repair. Applications of rhBMP-2 have utilised dosages between 5 μ g and 100 mg in large animal studies (Gu *et al.*, 2011; Nilsson *et al.*, 1986), and 0.1 μ g and 5 mg in small animal models (Hayashi *et al.*, 2009; Whang *et al.*, 1998). A few small animal studies have also used higher dosages up to 150 mg (Dohzono *et al.*, 2009; Hou *et al.*, 2012). One explanation for the observed variable success and diverse dosages is the number of different size models and defects investigated. Large animal models included dog (Hussein *et al.*, 2012), goat (Li *et al.*, 2010b), horse (Tsuzuki *et al.*, 2012), monkey (Bai *et al.*,

2009), pig (Abbah *et al.*, 2011) and sheep (Gu *et al.*, 2011). Small animal models included mouse (Yu *et al.*, 2010b), rabbit (Liu *et al.*, 2013) and rat (Iyomasa *et al.*, 2012). Bone defects investigated included both endochondral and intramembranous bone segmental defects (Boerckel *et al.*, 2012; Kirker-Head *et al.*, 1998; Wikesjo *et al.*, 2008), lumbar fusions (Akamaru *et al.*, 2003; Fu *et al.*, 2009) and drill defects (He *et al.*, 2009; Levi *et al.*, 2010), in addition to ectopic implants intramuscularly (Luca *et al.*, 2010b; Saito *et al.*, 2003) and subcutaneously (Fu *et al.*, 2010; Kimura *et al.*, 2010). Consequently, it may be more informative for authors to reference concentration and volume within given defect dimensions rather than a simple dosage as a standard between models. This would aid comparison between studies and animals enabling direct assessment of dosage and defect regeneration correlation. However, employed rhBMP-2 concentrations vary considerably, from 1.6 μ g/mL to 1.5 mg/mL for large animals (Itoh *et al.*, 1998; Sheehan *et al.*, 2003), and 2 μ g/mL to 4 mg/mL for small animals (Bax *et al.*, 1999; Woo *et al.*, 2001). There does not appear to be any correlation between dosages and fold increase in bone formation or time to healing. Therefore, reporting both defect volume and implant volume would help comparison of studies. It is important to clarify distinctions between studies and that ideal comparisons would be made between identical animals and anatomic defect locations, of which to date there are not enough publications for statistical comparison.

Indirect administration of BMP-2

Further studies have utilised indirect delivery by viral transduction or non-viral transfection of rhBMP-2 to the bone defect site with a view to enabling sustained localised growth factor delivery. The two main avenues for application are either virus particles/plasmid vectors (Dupont *et al.*, 2012; Ishihara *et al.*, 2008), or transduced/transfected cells (Lazard *et al.*, 2011; Lin *et al.*, 2008). The former influences non-specific host cell-originated tissue regeneration, whilst the latter enables exogenous cell-derived tissue repair. Indirect delivery studies showed a maximum 9-fold increase in bone formation (Wang *et al.*, 2009), demonstrating reduced tissue repair in comparison to direct delivery. This may be due to under-dosing at the defect site due to inadequate uptake by endogenous cells or production by exogenous cells. Continuous production of rhBMP-2 at the defect site may also have had negative or limiting effects on bone formation, compared to a single dose in most direct delivery studies. Spatiotemporal delivery at the defect site is therefore of paramount importance for augmentation of defect regeneration.

Combination administration of BMP-2

The bone-healing cascade is a complex process whose effective recapitulation is dependent on an exquisite interplay between multiple growth factors (Grimes *et al.*, 2011). A number of *in vivo* studies have thus investigated the use of growth factors in combination rather than single factor application (Table 5). Wang *et al.* (2012) delivered 5 µg of BMP-2 and 5 µg of BMP-7 *via* an implanted collagen sponge within a minipig calvarial defect, and demonstrated a 1.5 fold increase in bone formation compared to either growth factor alone. Koh *et al.* (2008) also investigated BMP-2 and BMP-7 indirect delivery *via* implantation of transduced cells within a mouse calvarial defect. The study showed a maximum 2-fold increase in bone formation compared to individual growth factor administration. Combination with other factors also demonstrated increased bone formation; Fujimura *et al.* (2002) showed a maximum 3.3-fold increase with BMP-7 and FGF-2 treatment, compared to 1.7 fold with BMP-7 alone. Clearly, there are benefits to dual combinations over single factors. Other factors and compounds used in combination with rhBMP-2, whether directly or indirectly, included BMP binding protein (BBP) (Lee *et al.*, 2011), erythropoietin (Sun *et al.*, 2012a), FGF (Springer *et al.*, 2008), α4-integrin (Kumar *et al.*, 2010a), Runx2 (Lee *et al.*, 2010), TGF-β2 (Thorey *et al.*, 2011), tobramycin (Glatt *et al.*, 2009) and zoledronic acid (Doi *et al.*, 2011; Schindeler *et al.*, 2011). A number of studies have incorporated mesenchymal stem cells (MSCs) (Hou *et al.*, 2007; Kim *et al.*, 2009) providing a healthy inducible cell source within the defect site (Dawson *et al.*, 2014). Taken together these studies demonstrated between 1.1 and 4 fold (Hou *et al.*, 2007; Thorey *et al.*, 2011) increased bone formation and defect regeneration (Supplement 1). Interestingly, some studies have shown that combined growth factor delivery does not enhance bone formation in comparison to single growth factor application. Terella *et al.* (2010) and Springer *et al.* (2008) demonstrated no further enhancement of bone regeneration above controls with BMP-2 treatment in

combination with MSCs and FGF-2, respectively. Indeed, Egermann *et al.* (2006) revealed a significant systemic retardation of bone formation within sheep injected with BMP-2 expressing adenovirus. This negative effect may be due to *i*) the combination of growth factors chosen or, *ii*) inhibitory or competitive effects between the combinations selected. The growth factor most often used successfully in combination with BMP-2 remains VEGF, where studies have reported a 1.4 to 20 fold (Xiao *et al.*, 2011; Zhang *et al.*, 2011a) increase in bone formation. Co-administration with VEGF induced vessel ingrowth bringing endogenous cells to the defect site, which could be triggered by BMP-2 to differentiate towards the osteogenic lineage and deposit new bone matrix. Ultimately, the aim of combination treatment is to support and augment native healing processes, and to do so requires a specific spatiotemporal approach with select growth factors.

BMP-7/OP-1

BMP-7, also known as OP-1, constitutes another BMP family member routinely used in bone tissue engineering strategies (Supplementary Table 2). Many *in vivo* studies have utilised BMP-7 on the basis that its osteoinductive potential can drive enhanced bone defect regeneration (Kidder *et al.*, 2009; Lee *et al.*, 2013).

Direct administration of BMP-7

Analogous to BMP-2, BMP-7 has been employed in many large (baboon (Ripamonti *et al.*, 2001a), dog (Fukuroku *et al.*, 2007), goat (den Boer *et al.*, 2002), monkey (Cook *et al.*, 2002) and sheep (Cipitria *et al.*, 2013)) and small (minipig (Warnke *et al.*, 2006), mouse (Lee *et al.*, 2013), rabbit (Haidar *et al.*, 2010b) and rat (Haidar *et al.*, 2010a)) animals with variable increased bone formation and defect regeneration ranging between 1.25 and 8.3 fold (Blokhuys *et al.*, 2001; Salkeld *et al.*, 2001) and 1.1 and 29.5 fold (Hamdy *et al.*, 2003; Kidder *et al.*, 2009), respectively. One interesting study by Chen *et al.* (2006) demonstrated a staggering 96 fold increase in mineralised callus formation after just 2 weeks with high dose OP-1 (200 µg). The bone defect model used however was complicated by *Staph. Aureus* infection and results should be carefully interpreted. Success variability may be dosage dependent since direct administration of BMP-7 has ranged from 100 µg to 3.5 mg (Reichert *et al.*, 2012; Ripamonti *et al.*, 2000) in large animals (some studies employed 65 mg to 750 mg (Lind *et al.*, 2001; Salkeld *et al.*, 2001)) and 0.025 µg to 3.5 mg (Sampath *et al.*, 1992; Warnke *et al.*, 2006) in small animals. However, it remains to be ascertained whether this suggested correlation is positive (higher dose results in higher bone formation (Chen *et al.*, 2006; Haidar *et al.*, 2010b; Ripamonti *et al.*, 2000)), negative (higher dose results in lower bone formation (Cook *et al.*, 2005; Soballe *et al.*, 2004)) or whether it indeed exists (bone formation remains unaffected by dosage (Hamdy *et al.*, 2003; Leknes *et al.*, 2008)). Standardisation of a species dependent bone defect model of specific dimensions and anatomic location, rather than a simply stated 'critical sized' defect, would inevitably aid interpretation of *in vivo* data. In turn, this would also help comparison between studies regarding the efficacy of individual growth factors such as BMP-7

to drive osteogenic bone formation. The variety of defects currently investigated include segmental (Chen *et al.*, 2002; Forriol *et al.*, 2009; Reichert *et al.*, 2012), drill (Lee *et al.*, 2013; Zhang *et al.*, 2004), fusion (Blattert *et al.*, 2002; Grauer *et al.*, 2004; Magin and Delling, 2001) and ectopic implantation intramuscularly (Haidar *et al.*, 2010a; Spiro *et al.*, 2010) and subcutaneously (Sampath *et al.*, 1992; Wei *et al.*, 2007), which together emphasise the lack of and need for standardisation. Indeed, Takigami *et al.* (2007) showed that anatomic location of the defect affected the efficacy of implanted BMP-7 on bone regeneration, where treatment of 10 mm femoral defects at the proximal and distal ends demonstrated 1.5-fold increased and 1.4-fold decreased bone formation. Other interesting observations which warrant further investigation include non-augmentation of bone formation following BMP-7 treatment and altered structure of new bone. Mont *et al.* (2001) showed that bone formation was the same with or without BMP-7 administration on allograft. It is important therefore to choose the scaffold material carefully as endogenous factors within the graft matrix may have masked the effects of BMP-7. Lammens *et al.* (2009) also reported a lack of bone augmentation following BMP-7 administration on bone filler. Spiro *et al.* (2010) showed that diclofenac treatment altered BMP-7-induced bone structure, decreasing trabeculae number and increasing spacing. Encouragingly, alternative ways of controlling inflammation at the defect site include the addition of BMP-binding peptide (BBP), which demonstrated a 1.5-fold reduction (Lee *et al.*, 2011).

Indirect administration of BMP-7

An alternative delivery method for growth factor delivery, as previously discussed, is viral transduction or non-viral transfection. BMP-7 has been indirectly delivered through the use of viral particles (2×10^{10} in large animals (Zhang *et al.*, 2007), and 1.8 to 2.5×10^{11} in small animals (Dunn *et al.*, 2005; Zhang *et al.*, 2012b)) and transduced/transfected cells (1×10^5 to 5×10^7 in large animals (Chaofeng *et al.*, 2013; Zhu *et al.*, 2010), and 1×10^5 to 2×10^6 in small animals (Li *et al.*, 2010a; Zhang *et al.*, 2010c)). Zhang *et al.* (2007) demonstrated a 2-fold increase in bone formation after implantation of viral particles, whilst Zhu *et al.* (2010) demonstrated a 2.5-fold increase after implantation of transduced bone marrow stromal cells (BMSCs) into dog and goat, respectively. Interestingly, the same study by Zhu *et al.* showed that implantation of non-transduced BMSCs also increased bone formation, but to a lesser degree (1.5 fold). Clearly, addition of cells alone without modification or growth factor loading can enhance bone defect regeneration. Typically, small animal studies demonstrated a 1.03 to 5 fold (Li *et al.*, 2010a; Zhang *et al.*, 2012b) increase in bone formation, although one study by Hidaka *et al.* (2003) reported 21 fold increased bone formation. However, this study investigated spinal fusion compared to segmental defects investigated in lower fold increase studies.

Combination administration of BMP-7

Additional factors have been successfully utilised in combination with BMP-7 to aid bone regeneration.

Combination of BMP-7 with BMP-2 constitutes an additive approach where two osteogenic factors are hypothesised to further enhance the osteogenic outcome, whilst keeping individual dosages low (Koh *et al.*, 2008; Wang *et al.*, 2012). Alternatively, combinations with VEGF (Roldan *et al.*, 2010) or TGF- β 3 (Ripamonti *et al.*, 2010) constitute mutualistic approaches where the angiogenic factor induces vessel ingrowth into the defect (2 fold (Zhang *et al.*, 2010a)), the chondrogenic factor induces cartilaginous matrix production to fill the defect void, and the osteogenic factor induces resultant callus mineralisation and eventual bone formation (three lineages important for recapitulation of the *in vivo* bone healing cascade). Other factors and compounds used in combination with BMP-7 include BBP (Lee *et al.*, 2011), FGF-2 (Ma *et al.*, 2007), insulin-like growth factor 1 (IGF-1) (Yang *et al.*, 2010), pamidronate (Yu *et al.*, 2010a), PDGF (Zhang *et al.*, 2012a), PTH (1-34) (Morgan *et al.*, 2008), TGF- β 1 (Ripamonti *et al.*, 2001b) and thrombospondin 1 (TSP-1) (Gelse *et al.*, 2011). Taken together these combinations have demonstrated increased defect regeneration from 1.4 to 5.3 fold in large animals (Ripamonti *et al.*, 2010; Zhang *et al.*, 2009b), and 1.2 to 15 fold in small animals (Yang *et al.*, 2010; Zhang *et al.*, 2012a). Although enhanced bone formation was observed in most studies, fold increases were not superior to those investigating BMP-7 alone. This may be due to under or over-dosing of one or both of the delivered factors. Consequently, balance between combination choice and dosage should be carefully considered as one study demonstrated a 2-fold decrease in bone formation following high dose pamidronate (2 mg) compared to low dose (20 μ g) pamidronate (Yu *et al.*, 2010a). A number of studies have also investigated BMP-7 combinations with cells including BMSCs (Zhang *et al.*, 2011b), MSCs (Tsiridis *et al.*, 2007a) or osteoblasts (Reichert *et al.*, 2011). Rather than flood the defect site with copious exogenous growth factors, these approaches endeavoured to augment the effect of BMP-7 through addition of an inducible cell source, and demonstrated a 1.5- to 8.8-fold increase in bone formation (Reichert *et al.*, 2011; Takigami *et al.*, 2007). Again, fold increases were not superior to those of BMP-7 alone, which may be due to non-optimal balance between dosage and cell number, or even non-optimal spatiotemporal delivery of BMP-7.

It is important to note that the different outcomes observed within all the collated studies described here, with the administration of BMP-2 and BMP-7 *in vivo*, may not solely be dependent on dosage but also on receptor expression. Inter-species receptor expression can vary considerably and may ultimately govern the response to BMP dosage.

FGF

FGFs constitute a large growth factor family with over 20 members and are involved in many biological processes from embryonic development regulating cell proliferation, migration and differentiation, to homeostasis orchestrating tissue maintenance and repair (Ornitz and Itoh, 2001). FGF-1 to FGF-10 all bind FGF receptors (FGFR) and have characterised functions in bone development and healing (Ornitz and Marie, 2002). A review of the literature

revealed the most abundant member utilised within bone tissue engineering strategies *in vivo* was FGF-2, also known as basic FGF (Hirata *et al.*, 2013; Hong *et al.*, 2010; Maehara *et al.*, 2009; Shirakata *et al.*, 2010).

Direct administration of FGF

FGF-2 has been administered to large animals including dog (Murakami *et al.*, 2003) and primate (Takayama *et al.*, 2001) at dosages from 0.15 to 200 µg (Hosokawa *et al.*, 2000; Nakamura *et al.*, 1998), and small animals including mouse (Kodama *et al.*, 2009), rabbit (Nakasa *et al.*, 2008) and rat (Tsurushima *et al.*, 2010) at dosages from 0.01 to 200 µg (Komaki *et al.*, 2006; Zellin and Linde, 2000) (Supplementary Table 3). Considering related fold increases in bone formation within large (1.3 to 3 fold (Nakamura *et al.*, 1998; Shirakata *et al.*, 2010)) and small animals (1.1 to 16.4 fold (Goodman *et al.*, 2003; Hong *et al.*, 2010)), it is interesting to note that higher dosages correlated with greater fold increases (defects with highest fold increase included tibial fracture and calvarial defect respective to large and small animals). Evidently, data suggest positive correlation between FGF-2 treatment and bone formation, potentially due to induced vessel ingrowth and ossification at the defect site (Guo *et al.*, 2006; Maehara *et al.*, 2009). However, administering the correct dose relative to defect size and location is paramount, since Nakasa *et al.* (2008) demonstrated a 1.5 fold decrease in lamellar bone formation following administration of 100 µg FGF-2 to a 5 mm full thickness femoral condyle defect. Although lamellar bone tissue was reduced, vascularisation and osseointegration were elevated, indicating accelerated maturation of extant bone. Indeed Bland *et al.* (1995) also demonstrated callus maturation without augmentation of bone tissue formation. Interaction of FGF-2 and condylar tissue may, in this instance, have had predominant effects on chondrogenesis, rather than osteogenesis. Nakasa *et al.* (2005) also investigated ectopic delivery of FGF-2 (subcutaneous implantation in rabbit) and observed extensive osteoid deposition, suggesting that interactions between the delivered growth factor and surrounding tissues dictate outcome.

Compared to FGF-2, a handful of studies utilised FGF-1, also known as acidic FGF (Bland *et al.*, 1995; Dunstan *et al.*, 1999; Kelpke *et al.*, 2004), and one study utilised FGF-18 (Carli *et al.*, 2012) which has been shown to promote chondrogenesis amongst many other functions. FGF-1 was administered between 3 and 7 µg in small animals including mouse (Dunstan *et al.*, 1999), rabbit (Bland *et al.*, 1995) and rat (Kelpke *et al.*, 2004). Dunstan *et al.* (1999) demonstrated 8 to 10 fold increased bone formation. However, the animal model was ovariectomised to create a state of osteoporosis and therefore resultant data require careful interpretation prior to comparison with that of other animal models. Fold increase would be expected to drop in a normal animal model as baseline bone regeneration levels would be higher than those in osteoporotic models. The same study also investigated injection of FGF-1 adjacent to mouse calvaria, which demonstrated a 3-fold increase in bone thickness. However, injection with FGF-2 exhibited a 7-fold increase in bone thickness, suggesting FGF-2 is a more potent osteoinductor compared to FGF-1. Kelpke *et*

al. (2004) demonstrated increased osteogenesis assessed by alkaline phosphatase, osteocalcin and osteopontin expression; however, bone tissue formation was not reported. Increased blood vessel ingrowth was observed and reported to range between 2 and 2.6 fold. Augmentation of vasculature can be beneficial for bone regeneration as it supplies an endogenous inducible cell source to populate and repair the defect site. Carli *et al.* (2012) delivered 0.5 µg FGF-18 to a 5 mm segmental femoral defect and demonstrated a 5-fold increase in percentage bone volume. On first observation, this would suggest that FGF-18 is a potent osteoinductor; however, this is only one study and was tested within a mutated mouse model which showed impaired bone formation. Further study is required to draw conclusions regarding *in vivo* bone tissue formation efficacy of FGF-1 and FGF-18.

Indirect administration of FGF

FGF-2 has been indirectly delivered to both large (dog (Tan *et al.*, 2009)) and small animals (mouse (Meng *et al.*, 2012), rabbit (Guo *et al.*, 2006) and rat (Qu *et al.*, 2011)) through implantation of transfected cells (6.25 x 10⁴ and 5 x 10⁶ cells (Guo *et al.*, 2006; Meng *et al.*, 2012)). Bone formation was modestly increased 2 to 2.7 fold (Kwan *et al.*, 2011; Meng *et al.*, 2012). However, one study by Hall *et al.* (2007) reported a 53.5 fold increase in percentage cancellous bone area (0.4 % in the control group increased to 21.4 % in the FGF-2 treated group). As previously discussed, care should be taken when comparing this with other data as the animal model used was haematopoietic deficient resulting in decreased baseline healing and therefore elevated fold increases in comparison to normal animal models. Most direct administration studies deliver FGF-2 in a single dose direct to the defect site and may be encapsulated within a carrier for controlled release over time, whereas indirect administration through endogenous or exogenous cell expression leads to continuous FGF-2 delivery. Lower bone tissue augmentation observed within indirect administration studies may be a consequence of constant exposure to FGF-2 stimulation. A spatiotemporal release profile would be more suitable to bone tissue-engineering strategies eligible for clinical translation.

Combination administration of FGF

Investigation of other tissues would aid understanding of whether lower fold increase in bone tissue was due to preference or induction of pre-bone tissue formation, such as osteoid deposition or cartilage production. Indeed, Iwaniec *et al.* (2003) demonstrated 8 fold increased osteoid deposition compared to 1.8 fold increased bone formation rate. Combinations should be carefully selected, as Behr *et al.* (2012) demonstrated that combination of FGF-2 with either BMP-2 or VEGF resulted in less bone formation compared to BMP-2 and VEGF in combination. However, selection according to the task at hand, whereby combination treatment with FGF-2 may be required to induce void filling callus formation and osteoid production prior to combination treatment with BMP-2 for mineralisation. A staged approach may be necessary for efficient and effective bone healing requiring multiple growth factors delivered spatiotemporally in

varying dosages. Following combination administration of FGF-2 with BMP-2 (van der Stok *et al.*, 2013), BMP-7 (Ma *et al.*, 2007), oestrogen (Iwaniec *et al.*, 2003), IGF-1 (Madry *et al.*, 2010), melatonin (Takechi *et al.*, 2008), PTH (1-34) (Lane *et al.*, 2003a), VEGF (Behr *et al.*, 2012) or 17 β -estradiol (Lane *et al.*, 2003a) in small animal models, including mouse (Behr *et al.*, 2012), rabbit (Madry *et al.*, 2010) and rat (Nakamura *et al.*, 2005), 1.1 to 3.3 fold (Fujimura *et al.*, 2002; Lane *et al.*, 2003a) increased bone formation was observed. Dual combinations evidently have important ramifications for bone tissue formation and defect regeneration albeit at magnitudes smaller than FGF-2 delivery alone. It is difficult to interpret whether interactions between dual factors resulted in synergistic or antagonistic signalling. Indeed, it is well known that FGF-2 inhibits BMP-2-driven bone formation by interfering with the signal transduction pathway, demonstrating a need for careful co-administration, possibly in a spatiotemporal manner. Analysis of parameters other than bone may help elucidate the mechanisms in play and aid comparison between studies. Although all studies reported increased bone formation, many did not compare to either growth factor individually or investigate non-bone tissues, such as cartilage production. Comparison to single factors would distinguish between synergistic and antagonistic signalling (greater or lower fold increase following dual combination, respectively).

PDGF

PDGF is a potent mitogen for the induction of angiogenesis from progenitor cells of the mesenchymal lineage. There are 5 isoforms including PDGF-A, B, C, D and a heterodimer AB. Homodimer BB constitutes a dimeric glycoprotein of PDGF that has been administered *in vivo* within small animals, including the minipig (Herford and Cicciu, 2012), mouse (Ranly *et al.*, 2005), rabbit (Lee *et al.*, 2001b) and rat (Kaipel *et al.*, 2012) for bone tissue engineering strategies (Supplementary Table 4).

Direct administration of PDGF

Direct administration of PDGF *in vivo* has been shown to increase bone tissue formation and defect regeneration between 1.5 (Herford *et al.*, 2012) and 2.4 fold (Moore *et al.*, 2009), following dosages ranging from 0.01 (Ranly *et al.*, 2005) to 80 μ g (Nash *et al.*, 1994) (two studies have also employed dosages up to 750 μ g (Herford and Cicciu, 2012; Herford *et al.*, 2012)). One study by Park *et al.* (2000) demonstrated up to 10 fold increased bone formation; however, this was compared to healing within blank control defects. The same study demonstrated only a 1.5 fold increase in comparison to control defects filled with scaffold alone. Evidently, dependent on the controls used and comparisons made within individual studies, care should be taken when drawing conclusions regarding growth factor efficacy for bone regeneration *in vivo*. Careful consideration should also be afforded to which parameters are used to quantify growth factor efficacy, as Moore *et al.* (2009) reported 9 fold increased union but only 1.9 to 2.4 fold increased bone volume. Standardisation of comparable readouts across related

growth factor investigations would ultimately provide fast and efficient cross-evaluation. PDGF appears to enhance bone regeneration through angiogenic induction and augmentation of surrounding vasculature. However, where increased bone formation is reported following high dose PDGF by Nash *et al.* (1994) reduced mechanical strength within newly formed bone was also reported. Quality alongside quantity of newly formed bone should therefore be factored into any analysis of bone defect regeneration. Although many studies have reported a positive correlation between PDGF administration and enhanced bone healing, these observations were not shared by all. Kaipel *et al.* (2012) demonstrated the failure of PDGF treatment to increase bone healing within a femoral segmental defect in rat. The same study also demonstrated failed healing following administration of another angiogenic factor VEGF. Interestingly, administration of BMP-2 within this study enhanced bone healing, suggesting that osteogenic factors are either a prerequisite for bone augmentation, or that they are required to drive progression of endogenous endochondral ossification.

Indirect administration of PDGF

Anusaksathien *et al.* (2004) reported similar negative findings with continuous PDGF exposure where treatment resulted in reduced mineralisation at the defect site. Delivery was indirect, through implantation of transduced cells. However, reduction was observed after 3 weeks then reversed and increased after 6 weeks. This suggests that temporal exposure within a larger network of bone healing processes dictates the effect of implanted PDGF. Addition of an angiogenic factor may not necessarily correlate with an angiogenic response, and is dependent on spatiotemporal delivery. Indeed, indirect administration of PDGF has been shown to modestly increase bone volume within the defect site between 1.7 (Chang *et al.*, 2010) and 2 fold (Zhang *et al.*, 2012a) following delivery of 5.5×10^8 to 5.5×10^9 PFU/mL within rats. Clearly, there is interplay between the growth factor delivered and endogenous processes at the defect site, which ultimately control the response observed. It is therefore valuable to successful tissue engineering strategies, to investigate and compare these interactions.

Combination administration of PDGF

A number of studies have investigated combination treatment with PDGF and several other growth factors including bFGF (Meraw *et al.*, 2000), BMP-2 (Martino *et al.*, 2011), BMP-7 (Zhang *et al.*, 2009b), IGF-1 (Nociti Junior *et al.*, 2000), osteogenin (Marden *et al.*, 1993), TGF- β 1 (Reyes *et al.*, 2012) and VEGF (El Backly *et al.*, 2013). Bone formation was reported to increase between 1.4 and 2.3 fold (Zhang *et al.*, 2009b) within large animals (dog (Zhang *et al.*, 2009b)) following PDGF dosages around 5 μ g/mL for direct administration (Nociti Junior *et al.*, 2000), or 2×10^{10} mL viral particles for indirect administration (Zhang *et al.*, 2009b). Combination treatments within small animal (mouse (El Backly *et al.*, 2013), rabbit (Reyes *et al.*, 2012) and rat (Park *et al.*, 2013)) studies reported increased bone formation between 2.5 (Xu

et al., 2012) and 10 fold (Reyes *et al.*, 2012) following PDGF dosages ranging between 0.05 (Martino *et al.*, 2011) and 200 μg (Marden *et al.*, 1993).

PTH

PTH is an 84 amino acid polypeptide secreted by chief cells of the parathyroid gland and is an essential regulator of both calcium and phosphate metabolism which has important ramifications for bone. Regarding mineral homeostasis, PTH acts to increase serum calcium through gastrointestinal absorption, renal reabsorption and liberation from bone reserves (Alkhiary *et al.*, 2005; Podbesek *et al.*, 1983). Continuous PTH treatment results in bone resorption, functioning indirectly through osteoblasts rather than directly *via* osteoclasts. However, intermittent PTH treatment has been shown to result in osteoblast stimulation and increased bone formation (Hock and Gera, 1992).

Direct administration of PTH

Many truncated forms of PTH have been directly administered *in vivo* most often by subcutaneous injection within large (dog (Daugaard *et al.*, 2012), monkey (Vahle *et al.*, 2008) and sheep (Arrighi *et al.*, 2009)) and small animals (mouse (Takahata *et al.*, 2012), rabbit (Lehman *et al.*, 2010) and rat (Qiu *et al.*, 2013)) at variable dosages from 0.75 to 7.5 $\mu\text{g}/\text{kg}/\text{day}$ (Manabe *et al.*, 2007) (20 to 1,000 $\mu\text{g}/\text{mL}$ (Arrighi *et al.*, 2009; Jung *et al.*, 2007a)), and 0.05 to 800 $\mu\text{g}/\text{kg}/\text{day}$ (Mohan *et al.*, 2000; Rihani-Bisharat *et al.*, 1998) (20 to 100 $\mu\text{g}/\text{mL}$ (Jung *et al.*, 2007b)), respectively (Supplementary Table 5). Treatment resulted in enhanced bone formation between 1.1 and 3.4 fold (Arrighi *et al.*, 2009; Vahle *et al.*, 2008) within large animals, and between 1.1 and 13.1 fold (Komatsu *et al.*, 2009; Li *et al.*, 2001) in small animals. Respective bone mechanical strength was also increased ranging from 1.4 to 2 fold (Daugaard *et al.*, 2011; Vahle *et al.*, 2008) for large animals and 1.1 to 3.8 fold (Reynolds *et al.*, 2011; Sloan *et al.*, 2010) for small animals. Thus teriparatide (PTH 1-34), the truncated PTH molecule, is a successful osteoporosis molecule with clear anabolic bone formation activity. In brief, PTH administration leads to increased bone formation and mechanical strength over time, possibly through a reduction in osteoclast number (Manabe *et al.*, 2007). Indeed, Nozaka *et al.* (2008) reported a 5.3-fold reduction in osteoclast number. However, other studies by O'Loughlin *et al.* (2009) and Takahata *et al.* (2012) reported contrasting results with a 2.5- to 4-fold increase in osteoclast number. Markers of bone formation such as osteocalcin and alkaline phosphatase were also shown to be increased between 1.2 and 3.1 fold (Komrakova *et al.*, 2011; Qiu *et al.*, 2013), indicating upregulation of osteoblast activity. PTH-enhanced osteoblast activity has been shown to reduce periodontal disease-induced bone loss by as much as 2.3 fold (Marques *et al.*, 2005). Conversely, continuous PTH infusion, investigated by Ma *et al.* (2001), demonstrated a significant drop in bone formation markers (3 to 7.5-fold drop in osteoprotegerin which binds RANKL blocking RANK-induced osteoclastogenesis) and increase in RANKL expression (5.5 to 27 fold) leading to a 3 fold increase in osteoclast number. Consequently, the adopted

administration regimen has significant implications for bone formation. Vahle *et al.* (2004; 2008) showed treatment withdrawal reversed bone enhancement after 3 years in sheep and 24 months in rat. Caution should therefore be taken when striking a balance between treatment period and, importantly, dosage, as Vahle *et al.* (2004) also demonstrated bone neoplasia with high dose PTH over prolonged periods in rats. However, the delivery vehicle may aid beneficial outcomes from continuous PTH administration, since Arrighi *et al.* (2009) demonstrated a maximum 3.4-fold increase in bone formation within sheep femoral and humeral defects following PTH fusion protein within fibrin glue. One interesting observation which may need future consideration for comparative purposes is the source of PTH under investigation. Li *et al.* (2001) reported a significant difference in the potency of two differently sourced PTH peptides, where bovine PTH was 4 to 6 fold more potent than rat PTH. As previously mentioned, some studies have investigated cartilage formation as a precursor to bone tissue generation. Following PTH treatment chondrogenesis/cartilage formation was increased 3 to 9.9 fold (Kakar *et al.*, 2007; O'Loughlin *et al.*, 2009) leading to enhanced trabeculated callus formation (Reynolds *et al.*, 2011). Bone architecture and structure are important quality indicators, yet many studies report only simple measurements of bone quantity.

Combination administration of PTH

PTH has been used in combination treatment of bone defects with growth factors including BMP-2 (Kempen *et al.*, 2010), BMP-7 (Morgan *et al.*, 2008), FGF-2 (Lane *et al.*, 2003b), IL-6 (Rozen *et al.*, 2007) and PTHrP (Xue *et al.*, 2005), and bisphosphonates including alendronate (Campbell *et al.*, 2011), ibandronate (Yang *et al.*, 2013), pamidronate (Aspenberg *et al.*, 2008), tiludronate (Delmas *et al.*, 1995) and zoledronic acid (Li *et al.*, 2013). Some studies have delivered PTH with cells, including periodontal ligament cells (Wolf *et al.*, 2012), BMSCs (Pettway *et al.*, 2005) and MSCs (Yu *et al.*, 2012b). Together, these studies have demonstrated increased bone formation between 1.3 (Morgan *et al.*, 2008) and 4.1 fold (Kempen *et al.*, 2010) in small animals following dosages from 10 to 90 $\mu\text{g}/\text{kg}/\text{day}$. One large animal study demonstrated between 2 and 4 fold following dosages of 0.015 $\mu\text{mol}/\text{kg}$ (Delmas *et al.*, 1995). Whilst bone formation was enhanced, it is important to note here that the same selection of studies together demonstrated enhanced bone formation between 1.2 (Niziolek *et al.*, 2009) and 3.1 fold (Pettway *et al.*, 2008) following treatment with PTH alone. Combination treatment also augmented mechanical parameters of new bone tissue including strength and stiffness between 1.2 (Morgan *et al.*, 2008) and 3.1 fold (Rozen *et al.*, 2007). Wolf *et al.* (2012) demonstrated 1.2 to 3 fold increased bone marker expression. One interesting study by Niziolek *et al.* (2009) delivered PTH with the antibiotic rapamycin and demonstrated reduced bone mineral density (BMD). This study highlights the need to standardise drug regimen between animal models as antibiotics are often administered following surgery and defect preparation. Drug selection should be carefully considered so as not to hinder effective defect regeneration.

PTHrP

As a regulator of endochondral bone development, PTHrP maintains growth plate width and structure through balanced inhibition of chondrocyte maturation (Kobayashi *et al.*, 2002). In adults, PTHrP interferes with osteocyte-mediated sclerostin inhibition of bone formation (Robling *et al.*, 2008), and binds receptors of the osteoblast lineage inducing enhanced bone formation (Karaplis, 2001).

Direct administration of PTHrP

PTHrP analogues and truncated peptides have been utilised directly in small animal models, including mouse (Lozano *et al.*, 2010), rabbit (Trejo *et al.*, 2010), and rat (Stewart *et al.*, 2000) (Supplementary Table 6). Bostrom *et al.* (2000) injected the PTHrP analogue RS-66271 within a rabbit ulna segmental defect (1 mm), whilst Trejo *et al.* (2010) implanted the C-terminal PTHrP (107-111) epitope within a rabbit femur epiphyseal defect (5 mm). These two studies demonstrated a 2 to 10 fold increase in bone volume (Table 5). Analogues and truncated peptides have also been assessed in disease models. The C-terminal PTHrP (107-139) peptide (Lozano *et al.*, 2010) and N-terminal PTHrP (1-36) peptide (Lozano *et al.*, 2009) were examined within diabetic mice and found to reverse diabetic-induced bone loss when administered at 100 µg/kg every other day. Interestingly, the N-terminal PTHrP (1-36) peptide (40 µg/kg/day) and PTHrP analogue RS-66271 (80 µg/kg/day) were investigated in ovariectomised rats (osteopaenia model) and found to reverse bone loss and enhance new bone formation exhibiting increased (3 fold) biomechanical strength (Stewart *et al.*, 2000; Vickery *et al.*, 1996).

Combination administration of PTHrP

Porto-Nunez *et al.* (2010) and de Castro *et al.* (2011) both used the N-terminal PTHrP (1-36) and the C-terminal PTHrP (107-139) peptides in combination in the ovariectomised and diabetic mice, respectively. Following injections at 80 and 100 µg/kg, bone loss was reversed and bone volume increased 1.5 fold. Although, different dosage regimes were implemented between these two studies, both observed an increase in BMD; 1.1 and 2.2 fold. Evidently, PTHrP plays an important role in bone formation and use of active analogues and peptides will not only augment bone healing, but can also reverse bone loss due to disease.

TGF-β3

A central component of the healing cascade in any bone defect is the formation of cartilage tissue, a precursor to immature bone, which subsequently becomes mineralised (Dimitriou *et al.*, 2005). TGF-β3 is a potent chondrogenic growth factor enhancing hyaline cartilage formation *in vivo* (Ripamonti *et al.*, 2009a; Tang *et al.*, 2009).

Direct administration of TGF-β3

Ripamonti *et al.* (2009b; 2008) and Teare *et al.* (2008) investigated the direct delivery of TGF-β3 (5 to 125 µg) within adult Chacma baboons and demonstrated a 1.75- to 3-fold increase in bone formation (Supplementary Table 7). Direct TGF-β3 delivery (3 ng to 2.75 µg) within small animal models, including mouse (Kovacevic *et al.*, 2011) and rat (Opperman *et al.*, 2002), only showed a 1.05 fold

increase in bone volume. However, cartilage formation showed a more robust augmentation of 1.23 fold (Table 5). Low levels of bone formation was also observed by Rizk and Rabie (2013), following investigation of TGF-β3 transduced cells within a mouse ectopic subcutaneous implant model; considerable cartilage constructs were generated without significant bone formation. Release of the chondrogenic factor TGF-β3 *in vivo* would be anticipated to induce cartilage formation. An appropriate osteogenic signal would then be required to drive mineralisation of this induced cartilage. Indeed, addition of osteogenic protein 1 (OP-1) (Ripamonti *et al.*, 2010) or MSCs (Mrugala *et al.*, 2008) within large animal models have been shown to increase bone formation 5.3 fold; a vast improvement over TGF-β3 alone.

Combination administration of TGF-β3

Small animal studies have investigated TGF-β3 delivery in combination with BMP-2 (Oest *et al.*, 2007), chondrocytes (Park *et al.*, 2010b), MSCs (Park *et al.*, 2010a), Sox9 (Park *et al.*, 2012), and TGF-β1 (Kim *et al.*, 2010a). Between 10 and 100 ng/mL TGF-β3 was used within these studies and TGF-β3 in combination with BMP-2 induced 12.8 to 13 fold more bone (Oest *et al.*, 2007; Simmons *et al.*, 2004) where combination with chondrocytes induced 1.6 to 22 fold increased collagen (Na *et al.*, 2006; Park *et al.*, 2009). These studies confirm the combination of chondrogenic and osteogenic factors in a defined spatiotemporal pattern can lead to more enhanced bone tissue formation than application of TGF-β3 alone.

VEGF

VEGF constitutes a sub-family comprised of 5 members (VEGF-A to VEGF-D, and placental growth factor). VEGF-A is the most important of these members with a significant role in both vasculogenesis (*de novo* vasculature formation) and angiogenesis (vessel formation sprouting from existing vasculature) (Byrne *et al.*, 2005). Hypoxia and necrosis are major concerns at sites of bone damage and contribute to healing failure. Formation of healthy vasculature through the use of VEGF-A to supply oxygen and nutrients at these sites is of paramount importance to efficient bone defect regeneration (Geiger *et al.*, 2007).

Direct administration of VEGF

Currently only a limited number of studies have utilised VEGF for *in vivo* bone defect regeneration (Supplementary Table 8). Kanczler *et al.* (2008) implanted 1.7 µg rhVEGF₁₆₅ on poly(lactic acid) (PLA) scaffold within a mouse femur 5 mm segmental defect. After 4 weeks, augmentation of blood vessel formation was observed alongside a 1.65-fold increase in bone volume (Table 5). The same study implanted human BMSCs in combination with rhVEGF₁₆₅ but observed no further enhancement. A study by Wernike *et al.* (2010) observed enhanced vascularisation but negligible impact on bone regeneration within a mouse calvarial 4 mm drill defect.

Indirect administration of VEGF

A number of studies have investigated indirect delivery of VEGF₁₆₅ through implantation of plasmid DNA within

a mouse femur 8 mm defect (Keeney *et al.*, 2010), or transfected cells within rabbit long bone 10 mm and 15 mm segmental defects (Geiger *et al.*, 2007; Li *et al.*, 2009b). Together these studies demonstrated 1.6- to 2-fold increase in bone formation with augmented vascularisation.

Combination administration of VEGF

A number of studies have explored the application of angiogenic VEGF (0.2 to 20 µg) and osteogenic BMP-2 (0.5 to 120 µg) in combination within large animals, including dog (Geuze *et al.*, 2012) and pig (Ramazanoglu *et al.*, 2011), and small animals, including mouse (Behr *et al.*, 2012; Samee *et al.*, 2008), rabbit (Hernandez *et al.*, 2012), and rat (Kempen *et al.*, 2009). Roldan *et al.* (2010) and Zhang *et al.* (2010a) combined VEGF with BMP-7 and observed neovascularisation in the absence of any significant increase in bone regeneration. Li *et al.* (2009a) investigated the use of VEGF with BMP-4 and observed impaired ectopic bone formation using a high VEGF ratio. Interestingly, when VEGF release was slow and sustained, impairment was no longer observed. Recruitment of blood vessels into the defect site, instructed by VEGF, typically complicates bone formation due to the increased localised bone remodelling and callus formation by osteoblasts. Zhang *et al.* (2011a) found that VEGF delivery using a hydrogel resulted in faster degradation, which ultimately has repercussions for controlled dual growth factor release profiles. It is thus self-evident that spatiotemporal control of the select growth factor release for induction of angiogenesis, chondrogenesis, and osteogenesis is central for successful bone tissue repair.

Wnt Proteins

A diverse family of signalling glycoproteins (19 members; Wnt1 to Wnt16), Wnt proteins are involved in a myriad of cellular processes, including cell proliferation, migration and differentiation (De Boer *et al.*, 2004).

Direct administration of Wnt proteins

Zhou *et al.* (2009) injected 100 ng Wnt3A into a mouse model of delayed skeletal development and observed both increased parietal bone volume and a 1.4 fold reduction in suture area (Supplementary Table 9). Only one other study, at the time of writing this review, had investigated Wnt3A utilisation, using liposomal vesicle injection for direct delivery of Wnt3A to 1 mm tibial mouse fracture model (Minear *et al.*, 2010).

Indirect administration of Wnt proteins

Given the cost of Wnt proteins, focus has centred on indirect delivery using transduced and transfected cells (Table 5). Nalesso *et al.* (2011) and Qiang *et al.* (2008) both injected Wnt3A-transfected cells within severe combined immuno deficient (SCID) mice and observed a 1.5 fold increased cartilage formation and a 1.12 fold increased BMD. Liu *et al.* (2009) injected Wnt1 transduced cells within SCID mice and observed a dose dependent enhancement of bone formation (1.25 fold). Implantation of Wnt4 transduced MSCs in SCID mice with a 5 mm calvarial defect resulted in extensive integrated enhanced mineralised bone tissue (Chang *et al.*, 2007). The same

study implanted Wnt1 transduced cells within an alveolar defect in SCID rats and observed a 3- to 5-fold increase in bone formation. 1.75 fold increased bone formation was also observed by Bennett *et al.* (2005; 2007) within transgenic mice following Wnt10B plasmid injection into mouse embryos. These different studies indicate that Wnt proteins can augment *in vivo* bone formation, although success in bone tissue engineering will be dependent on Wnt protein selection. Injection of Wnt6 transfected cells within the chick limb bud inhibited chondrogenesis and promoted myogenesis (Geetha-Loganathan *et al.*, 2010). Wnt5A plasmid injection within mouse embryos generated transgenic mice exhibiting a variety of developmental defects, including reduced endochondral and intramembranous bone formation (van Amerongen *et al.*, 2012), although control of spatiotemporal expression exhibited increased calvarial ossification.

In summary, select exogenous factors can be successfully applied as part of a tissue-engineering regimen for *in vivo* bone regeneration. It is the opinion of the authors that BMP-2 provides the greatest bone regeneration *in vivo*, and that careful spatiotemporal release with additional factors may provide synergistic or additional signalling leading to further augmentation. Supporting literature discussed here details a maximum 50-fold increase in bone formation following BMP-2 administration (Table 5). BMP-7 may provide a suitable alternative to BMP-2 with similar osteogenic potency. However, many studies failed to quantify enhanced tissue formation or failed to record any changes therein and it may be that the recorded fold increases may indeed be higher. Nevertheless, these animal studies have informed clinical translation resulting in BMP-2, BMP-7/OP-1, PDGF, PTH and PTHrP transition from animals to humans. The current prohibitive protein production costs or minimal supporting *in vivo* literature may explain the lack of FGF, TGF-β3, VEGF and Wnt protein clinical translation to date.

Human Trials

In contrast to animal models, human patients display unpredictable idiopathic variations in their ability to form bone, inter and intra-family genetic variations, and systemic multifactorial inconsistencies derived from age, sex, weight, diet, disease, health status, lifestyle, medication, drug abuse/addiction, and numerous environmental factors (Sandhu *et al.*, 1995). Consequently, large subject numbers are required in any clinical trial before examination can yield comparative data of value (Khan and Lane, 2004). That said, tissue engineering-based approaches to bone regeneration in humans have already been successfully translated.

BMP-2

Approval for the use of rhBMP-2 in humans was granted by the European Medicines Agency (EMA) in 2002, and by the U.S. Food and Drug Administration (FDA) in 2004 (McKay *et al.*, 2007), following a pivotal study by the BESTT (BMP-2 evaluation in surgery for tibial trauma) study group (Govender *et al.*, 2002). The study

reported both a reduced need for secondary intervention, and enhanced fracture healing following treatment with rhBMP-2 on absorbable collagen sponge (ACS). BMP-2 has subsequently become the subject of intense examination *in vivo* (Supplementary Table 1). Clinical studies have included facial reconstruction (cleft and mandible defects) (Cicciu *et al.*, 2012; Dickinson *et al.*, 2008; Herford and Boyne, 2008), maxillary sinus floor augmentation (Triplett *et al.*, 2009), long bone non-unions (Tressler *et al.*, 2011), tibial fractures (Jones *et al.*, 2006; Swiontkowski *et al.*, 2006), and lumbar fusions (Mladenov *et al.*, 2010; Taghavi *et al.*, 2010). Facial reconstruction and bone augmentation studies all delivered rhBMP-2 on ACS at a concentration of 0.75 to 1.5 mg/mL. Boyne *et al.* (2005) demonstrated increased bone formation suitable for dental implants. Fiorellini *et al.* (2005) and Triplett *et al.* (2009) performed similar dental studies revealing 2-fold increase in bone formation for dental implants and functional longevity, respectively. Dickinson *et al.* (2008) also demonstrated the efficacy of rhBMP-2/ACS for bone regeneration *in vivo* through improved healing and reduced morbidity in cleft defects. Treated patients exhibited 95 % closure compared to 63 % in non-treated patients. New bone formation and closure of non-union fractures was observed by Tressler *et al.* (2011) and Johnson *et al.* (1988b). Additional advantages of utilising rhBMP-2 on ACS over iliac crest autograft included a 1.35 fold reduced operative time and 1.4 fold reduced intraoperative blood loss, both of which aided effective surgery (Tressler *et al.*, 2011). 92.3 % to 98 % of treated patients (Burkus *et al.*, 2009; Haid *et al.*, 2004) compared with 70 % to 89 % of control patients (Dawson *et al.*, 2009; Dimar *et al.*, 2009) exhibited successful fusions of lumbar vertebrae demonstrating enhancement of rhBMP-2-induced bone formation (Supplementary Table 1). Other rhBMP-2 studies reported reduced back and leg pain (Burkus *et al.*, 2003a) and reduced arm and neck pain (Baskin *et al.*, 2003). The major carrier utilised for rhBMP-2 was ACS, however alternative carriers were utilised including autograft and allograft bone (Buttermann, 2008; Taghavi *et al.*, 2010), gelatin (Johnson *et al.*, 1988a), hydroxyapatite-tricalcium phosphate (HA-TCP) particles (Dawson *et al.*, 2009), polyetheretherketone (PEEK) (Klimo and Peelle, 2009), and poly(lactic co-glycolic acid) (PLGA) (Johnson *et al.*, 1988b; Katayama *et al.*, 2009).

However, in the last few years a number of studies have cast a long shadow on the clinical efficacy (successful adverse-free outcomes) of rhBMP-2 for spinal fusion. Off label use of rhBMP-2, reported within several studies, has shown significant rhBMP-2-related side effects including urogenital complications, wound complications, increased inflammation and increased cancer risk. Furthermore, a number of reviews indicate an apparent failure of early journal publications to report, or under report, associated complications and selective individual patient reporting. These reviews also indicate misrepresentation of efficacy, together with duplicate publications (Carreon *et al.*, 2008; Fu *et al.*, 2013; Mesfin *et al.*, 2013; Moshel *et al.*, 2008).

BMP-7/OP-1

Early studies utilising BMP-7 in humans were first reported between 1999 and 2001 (Friedlaender *et al.*, 2001; Geesink *et al.*, 1999; Laursen *et al.*, 1999; van den Bergh *et al.*, 2000). These studies investigated BMP-7 delivery on ACS to long bone osteotomy and non-union, lumbar interbody fusion and maxillary sinus augmentation (Supplementary Table 2). Van den Bergh *et al.* (2000) and Groeneveld *et al.* (1999) reported 1.2 to 9.7 fold increased osteoid formation following treatment with 2.5 mg BMP-7 for maxillary sinus augmentation. Less successful outcomes were reported by Laursen *et al.* (1999) and Jeppsson *et al.* (1999) regarding lumbar fusions, where enhanced bone resorption was observed and only 1 of 4 patients exhibited successful bone bridging. Conversely, Geesink *et al.* (1999) demonstrated new bone formation within tibial osteotomies as early as 6 weeks following treatment with 2.5 mg BMP-7 in all but one patient. Friedlaender *et al.* (2001) demonstrated safe application of BMP-7 *in vivo* with non-union healing comparable to autograft controls. Together, these studies pioneered BMP-7 use *in vivo* and led to FDA approval for use in long bone non-unions in 2001 and posterolateral lumbar fusions in 2004 (Ong *et al.*, 2010). Regarding lumbar fusion surgery, BMP-7 was delivered at 3.5 mg per vertebral side (7 mg in total). Vaccaro *et al.* (2003; 2004; 2005) reported improved Oswestry scores measure low back pain, radiographic fusion in 50 % to 55 % of patients, and bone bridging in 70 % to 91 % of patients. BMP-7 was repeatedly shown to increase bone formation similar to autograft (Johnsson *et al.*, 2002; Kanayama *et al.*, 2006; Vaccaro *et al.*, 2004). However, over-zealous application of BMP-7 can have side effects, as Kim *et al.* (2010b) demonstrated significant ectopic bone formation along the surgical track following delivery of 17.5 mg. It is important to note that dosage is relative to defect site and that where high dose in one anatomic location may be excessive, in another location within a different size defect the same dose may be more appropriate. Indeed, 17.5 mg appeared excessive in lumbar fusion, but Hernandez-Alfaro *et al.* (2012) demonstrated safe administration of 2 g BMP-7 within a 60 mm mandibular defect and reported stable osseointegration of titanium mesh implant after 1 year. Numerous pseudarthrosis and non-union fracture studies standardised BMP-7 dosage to 3.5 mg. Complete defect healing was observed in patients treated for pseudarthrosis, where treated bones were reportedly pain free and load bearing (Anticevic *et al.*, 2006; Fabeck *et al.*, 2006). However, Lee *et al.* (2006) did not observe new bone formation in any of the 5 patients that received treatment. A study by Ekrol *et al.* (2008) also demonstrated a lack of BMP-7 induced bone healing within radial osteotomies reporting decreased healing rates and reduced stability compared to autograft. Non-union fracture studies reported better results following administration of 3.5 mg BMP-7. 75 % (Friedlaender *et al.*, 2001) to 100 % (Giannoudis *et al.*, 2009) of treated patients exhibited healed defects within 3 (Kanakaris *et al.*, 2008) to 16 months (Giannoudis *et al.*, 2009). This compared with only 68.3 % (Calori *et al.*, 2008)

to 85 % (Friedlaender *et al.*, 2001) of patients who received autograft. These studies also reported decreased healing time (1.5 fold), hospital stay (3.4 fold) and treatment cost (1.9 fold) (Dahabreh *et al.*, 2007; Ristiniemi *et al.*, 2007). Taken together, current literature supports the application of BMP-7 within tissue engineering strategies for localised bone defect repair and regeneration. However, dosage should be carefully considered with respect to the defect site to reduce unwanted side effects.

PDGF

Only one study, at the time of writing, was found to utilise PDGF for *in vivo* bone regeneration in humans. Nevins *et al.* (2003) administered between 0.5 and 5 mg/mL PDGF-BB to patients with advanced periodontitis and interproximal intrabony and/or molar class II furcation defects (Supplementary Table 4). As with all human studies, investigation of new bone is limited and analysis of specific bone parameters is restricted to non-invasive techniques. The study assessed defect regeneration through vertical probing depth and found a 1.12 fold reduction following PDGF treatment compared to xenograft bone in collagen. Clearly, PDGF treatment provides a functional alternative to xenograft for effective defect regeneration.

PTH

PTH has been administered in humans for many years investigating its efficacy for bone formation within healthy adults (Horwitz *et al.*, 2011), adults with low BMD (Ryder *et al.*, 2010), postmenopausal women (Schafer *et al.*, 2013), mandibular defects (Kwon *et al.*, 2012) and vertebral fractures (Nakamura *et al.*, 2012) (Supplementary Table 5). Continuous delivery has been shown to reduce bone formation markers and increase bone resorption. Horwitz *et al.* (2011) indeed demonstrated extensive bone resorption with high dose PTH (4 pmol/kg/h) delivered by continuous infusion pump, resulting in hypercalcemia. Intermittent delivery increased bone turnover within low BMD patients who exhibited 2.1 fold increased bone formation, and 2.7 fold increased bone resorption (Ryder *et al.*, 2010). Nakamura *et al.* (2012) also demonstrated increased BMD (2.3 to 6 fold) within vertebral fracture patients following intermittent administration (56.5 µg/week). Standard delivery of PTH at 20 µg/d to patients with mandibular defects resulted in 5.4 to 5.7 fold increased bone marker expression (Kwon *et al.*, 2012), and 1.5 (Kuchler *et al.*, 2011) to 11.6 fold (Bashutski *et al.*, 2010) increased bone formation with augmented implant integration (1.2 to 4.7 fold). Most studies reviewed investigated PTH administration within postmenopausal women at dosages ranging between 20 and 100 µg/d. These studies reported reduced healing time (1.3 (Aspenberg *et al.*, 2010) to 1.6 fold (Peichl *et al.*, 2011)) and fracture incidence (2.8 to 7 fold) (Neer *et al.*, 2001), whilst BMD and mechanical strength were reportedly increased 1.02 to 1.05 fold (Keaveny *et al.*, 2012), and 4.2 to 7.7 fold (Keaveny *et al.*, 2008), respectively. Clearly, PTH can augment bone defect repair and increase innate BMD.

PTHrP

PTHrP has been used clinically *via* subcutaneous or intravenous injection. Horwitz *et al.* (2011; 2005) published data detailing systemic delivery of N-terminal PTHrP (1-36) peptide within healthy adults at dosages between 2 and 28 pmol/kg/h led to profound suppression of bone formation (Supplementary Table 6). Another study revealed that 1.3- to 1.4-fold suppression in bone formation could be reversed following PTHrP analogue cessation. Consequently, continuous infusion can enhance bone resorption and decrease bone formation, whilst intermittent infusion can lead to a net increase in bone formation. Fraher *et al.* (1992) demonstrated increased serum calcium and urinary phosphate when healthy individuals were injected with N-terminal PTHrP (1-34) peptide at 8 or 80 pmol/kg/h. Thus, PTHrP translation from animal to human studies has, to date, not yielded similar responses, indeed the use of PTHrP analogues has had the opposite effect with increased bone resorption observed in the clinic. However, a study by Plotkin *et al.* (1998) using the N-terminal PTHrP (1-36) peptide delivered by subcutaneous injection within post-menopausal oestrogen deficient women observed activation of bone formation and a 1.3- to 1.45-fold reduction in bone resorption. The function of these analogues may be modified by the hosts hormonal status; pre or post-menopause. Minimal literature on the *in vivo* use of PTHrP analogues highlights the need for further investigation before definitive conclusions can be drawn.

Future directions

It is clear the use of select growth factors *in vivo* can augment bone formation and potentially repair defects. Utilisation of animal models has proven informative for clinical translation of bone tissue engineering strategies. However, complications associated with spatiotemporal release of growth factors regarding longevity, bioactivity and carrier release kinetics have impeded progress. Parallel, synergistic and consecutive delivery of multiple growth factors appears key to successful bone regeneration. The authors envisage coordinated spatiotemporal release of select growth factors recapitulating *in vivo* signalling cascades leading to bone tissue formation. The importance of understanding the developmental processes underpinning bone tissue formation, and their importance in contextualising signalling cascades and the growth factors involved in regenerative medicine is gaining prominence, as understanding these processes is vital to informed clinical bone therapies (Smith *et al.*, 2013; Turner *et al.*, 2013). The data presented here demonstrate the complex and convoluted interplay between administered growth factors with variable success for bone tissue formation dependent on species, dosage and combination. Thus, before robust bone tissue engineering can be achieved (and more importantly interpreted), it will be important to understand the functional interplay between

growth factors and how this leads to bone formation under different conditions. For example, the chick model provides an ideal system for investigating bone development biology (Smith *et al.*, 2013). Organotypic culture of embryonic chick femora *ex vivo* enables investigation and elucidation of processes involved in skeletal development and bone repair (Kanczler *et al.*, 2012; Smith *et al.*, 2014a; Smith *et al.*, 2014b; Smith *et al.*, 2012). Models such as the chick may indeed fulfil the requirement for a simple, relatively high throughput and cost effective research tool with which to inform, create and optimise bone tissue engineering strategies.

Conclusions

Evaluation of the osteotropic factors presented here confirms the potential of these factors to augment bone formation *in vivo* cementing their selection for bone reparation. Current reports indicate that BMP-2 and BMP-7 have significant potential to augment bone formation (up to 50 fold and 96 fold, respectively) through induced osteogenesis and osseointegration of tissue-engineered implants. However, lessons from off-label complications and issues surrounding potential adverse events associated with rhBMP-2 in spinal fusion need to be carefully considered. In order, FGF, PTH, Wnt proteins, PTHrP analogues, PDGF, TGF- β 3 and VEGF have demonstrated up to 16.4, 13.1, 12, 10, 10, 3 and 2 fold increased bone formation following direct and indirect delivery. Although not as potent as BMPs, these growth factors clearly have important benefits in any tissue engineering strategy. Sequential release of these angiogenic, chondrogenic, and osteogenic factors recapitulating native environmental cues is undoubtedly critical to successful bone augmentation. Many studies have therefore investigated combinational growth factor delivery to further enhance bone regeneration. However, most combination treatments to date appeared to enhance bone formation to a lesser degree. In order, BMP-2, BMP-7, PTH, FGF and PTHrP demonstrated 20, 15, 4.1, 3.3 and 3 fold increased bone formation, respectively. TGF- β 3 and VEGF combination treatments conversely showed further enhancement with 13 and 20 fold increased bone formation. Important to note here is that both TGF- β 3 and VEGF were combined with BMP-2, which on the one hand improved their osteo-inductive potential and yet, apparently, diminished the osteo-inductive potential of BMP-2. Combinational PDGF treatments demonstrated similar augmentation to PDGF treatments alone. Wnt proteins were not found to have been used for combination treatment. Considering reported bone formation following combination treatment, it is evident that suboptimal spatiotemporal delivery and complicated *in vivo* interplay is hindering further enhancement. Further understanding the complex spatiotemporal interactions between growth factors *in vivo*, through use of appropriate animal models, will aid generation of clinically transferable and effective bone tissue engineering strategies. A number of studies have demonstrated successful bone tissue engineering in humans using the growth factors discussed here.

However, a lack of bone tissue quantification and adequate controls limits correlation between growth factor efficacy in animals and that in humans. This further highlights the need for standardised investigation with specified measurable parameters *in vivo*. Connecting observations in animal models to those in humans will, ultimately, further our understanding of growth factor induced bone formation. Controlled orchestration of clinically relevant and functional *in vivo* bone formation may finally deliver on the long heralded promise of bone regeneration for an increasingly aged population.

Acknowledgements

This work was supported by a strategic longer and larger grant (sLOLA) from the Biotechnology and Biological Sciences Research Council, UK-grant number BB/G010579/1 to AEH, KMS and ROCO. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

- Abbah SA, Lam CX, Ramruttun AK, Goh JC, Wong HK (2011) Fusion performance of low-dose recombinant human bone morphogenetic protein 2 and bone marrow-derived multipotent stromal cells in biodegradable scaffolds: a comparative study in a large animal model of anterior lumbar interbody fusion. *Spine* **36**: 1752-1759.
- Abe N, Lee YP, Sato M, Zhang X, Wu J, Mitani K, Lieberman JR (2002) Enhancement of bone repair with a helper-dependent adenoviral transfer of bone morphogenetic protein-2. *Biochem Biophys Res Commun* **297**: 523-527.
- Abe Y, Takahata M, Ito M, Irie K, Abumi K, Minami A (2007) Enhancement of graft bone healing by intermittent administration of human parathyroid hormone (1-34) in a rat spinal arthrodesis model. *Bone* **41**: 775-785.
- Abu-Serriah MM, Odell E, Lock C, Gillar A, Ayoub AF, Fleming RH (2004) Histological assessment of bioengineered new bone in repairing osteoperiosteal mandibular defects in sheep using recombinant human bone morphogenetic protein-7. *Br J Oral Maxillofac Surg* **42**: 410-418.
- Aerssens J, Boonen S, Lowet G, Dequeker J (1998) Interspecies differences in bone composition, density, and quality: potential implications for *in vivo* bone research. *Endocrinology* **139**: 663-670.
- Akamaru T, Suh D, Boden SD, Kim HS, Minamide A, Louis-Ugbo J (2003) Simple carrier matrix modifications can enhance delivery of recombinant human bone morphogenetic protein-2 for posterolateral spine fusion. *Spine* **28**: 429-434.
- Al-Zube L, Breitbart EA, O'Connor JP, Parsons JR, Bradica G, Hart CE, Lin SS (2009) Recombinant human platelet-derived growth factor BB (rhPDGF-BB) and beta-

tricalcium phosphate/collagen matrix enhance fracture healing in a diabetic rat model. *J Orthop Res* **27**: 1074-1081.

Aleksyniene R, Thomsen JS, Eckardt H, Bundgaard KG, Lind M, Hvid I (2009) Parathyroid hormone PTH(1-34) increases the volume, mineral content, and mechanical properties of regenerated mineralizing tissue after distraction osteogenesis in rabbits. *Acta Orthop* **80**: 716-723.

Ali MN, Kobayashi T, Tanaka M, Ohshima H, Ejiri S, Saito C (2012) Effects of intermittent parathyroid hormone treatment on new bone formation during distraction osteogenesis in the rat mandible. *Oral Surg Oral Med Oral Pathol Oral Radiol* **114**: e36-42.

Alkhiary YM, Gerstenfeld LC, Krall E, Westmore M, Sato M, Mitlak BH, Einhorn TA (2005) Enhancement of experimental fracture-healing by systemic administration of recombinant human parathyroid hormone (PTH 1-34). *J Bone Joint Surg Am* **87**: 731-741.

Alpaslan C, Irie K, Takahashi K, Ohashi N, Sakai H, Nakajima T, Ozawa H (1996) Long-term evaluation of recombinant human bone morphogenetic protein-2 induced bone formation with a biologic and synthetic delivery system. *Br J Oral Maxillofac Surg* **34**: 414-418.

Andreassen TT, Cacciabesta V (2004) Intermittent parathyroid hormone treatment enhances guided bone regeneration in rat calvarial bone defects. *J Craniofac Surg* **15**: 424-427.

Andreassen TT, Ejersted C, Oxlund H (1999) Intermittent parathyroid hormone (1-34) treatment increases callus formation and mechanical strength of healing rat fractures. *J Bone Miner Res* **14**: 960-968.

Andreassen TT, Willick GE, Morley P, Whitfield JF (2004) Treatment with parathyroid hormone hPTH(1-34), hPTH(1-31), and monocyclic hPTH(1-31) enhances fracture strength and callus amount after withdrawal fracture strength and callus mechanical quality continue to increase. *Calcif Tissue Int* **74**: 351-356.

Anticevic D, Jelic M, Vukicevic S (2006) Treatment of a congenital pseudarthrosis of the tibia by osteogenic protein-1 (bone morphogenetic protein-7): a case report. *J Pediatr Orthop B* **15**: 220-221.

Anusaksathien O, Jin Q, Zhao M, Somerman MJ, Giannobile WV (2004) Effect of sustained gene delivery of platelet-derived growth factor or its antagonist (PDGF-1308) on tissue-engineered cementum. *J Periodontol* **75**: 429-440.

Arrighi I, Mark S, Alvisi M, von Rechenberg B, Hubbell JA, Schense JC (2009) Bone healing induced by local delivery of an engineered parathyroid hormone prodrug. *Biomaterials* **30**: 1763-1771.

Ascenzi MG, Liao VP, Lee BM, Billi F, Zhou H, Lindsay R, Cosman F, Nieves J, Bilezikian JP, Dempster DW (2012) Parathyroid hormone treatment improves the cortical bone microstructure by improving the distribution of type I collagen in postmenopausal women with osteoporosis. *J Bone Miner Res* **27**: 702-712.

Aspenberg P, Wermelin K, Tengwall P, Fahlgren A (2008) Additive effects of PTH and bisphosphonates on the bone healing response to metaphyseal implants in rats. *Acta Orthop* **79**: 111-115.

Aspenberg P, Genant HK, Johansson T, Nino AJ, See K, Krohn K, Garcia-Hernandez PA, Recknor CP, Einhorn TA, Dalsky GP, Mitlak BH, Fierlinger A, Lakshmanan MC (2010) Teriparatide for acceleration of fracture repair in humans: a prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. *J Bone Miner Res* **25**: 404-414.

Bae JH, Song HR, Kim HJ, Lim HC, Park JH, Liu Y, Teoh SH (2011) Discontinuous release of bone morphogenetic protein-2 loaded within interconnected pores of honeycomb-like polycaprolactone scaffold promotes bone healing in a large bone defect of rabbit ulna. *Tissue Eng Part A* **17**: 2389-2397.

Bai B, Yin Z, Xu Q, Lew M, Chen Y, Ye J, Wu J, Chen D, Zeng Y (2009) Histological changes of an injectable rhBMP-2/calcium phosphate cement in vertebroplasty of rhesus monkey. *Spine* **34**: 1887-1892.

Baim S, Leslie WD (2012) Assessment of fracture risk. *Curr Osteoporos Rep* **10**: 28-41.

Baltzer AW, Lattermann C, Whalen JD, Wooley P, Weiss K, Grimm M, Ghivizzani SC, Robbins PD, Evans CH (2000) Genetic enhancement of fracture repair: healing of an experimental segmental defect by adenoviral transfer of the BMP-2 gene. *Gene Ther* **7**: 734-739.

Barnes B, Boden SD, Louis-Ugbo J, Tomak PR, Park JS, Park MS, Minamide A (2005) Lower dose of rhBMP-2 achieves spine fusion when combined with an osteoconductive bulking agent in non-human primates. *Spine* **30**: 1127-1133.

Barr T, McNamara AJ, Sandor GK, Clokie CM, Peel SA (2010) Comparison of the osteoinductivity of bioimplants containing recombinant human bone morphogenetic proteins 2 (Infuse) and 7 (OP-1). *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **109**: 531-540.

Bashutski JD, Eber RM, Kinney JS, Benavides E, Maitra S, Braun TM, Giannobile WV, McCauley LK (2010) Teriparatide and osseous regeneration in the oral cavity. *N Engl J Med* **363**: 2396-2405.

Baskin DS, Ryan P, Sonntag V, Westmark R, Widmayer MA (2003) A prospective, randomized, controlled cervical fusion study using recombinant human bone morphogenetic protein-2 with the CORNERSTONE-SR allograft ring and the ATLANTIS anterior cervical plate. *Spine* **28**: 1219-1224.

Basmanav FB, Kose GT, Hasirci V (2008) Sequential growth factor delivery from complexed microspheres for bone tissue engineering. *Biomaterials* **29**: 4195-4204.

Bax BE, Wozney JM, Ashhurst DE (1999) Bone morphogenetic protein-2 increases the rate of callus formation after fracture of the rabbit tibia. *Calcif Tissue Int* **65**: 83-89.

Becker W, Lynch SE, Lekholm U, Becker BE, Caffesse R, Donath K, Sanchez R (1992) A comparison of ePTFE membranes alone or in combination with platelet-derived growth factors and insulin-like growth factor-I or demineralized freeze-dried bone in promoting bone formation around immediate extraction socket implants. *J Periodontol* **63**: 929-940.

Behr B, Sorkin M, Lehnhardt M, Renda A, Longaker MT, Quarto N (2012) A comparative analysis of the

osteogenic effects of BMP-2, FGF-2, and VEGFA in a calvarial defect model. *Tissue Eng Part A* **18**: 1079-1086.

Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, MacDougald OA (2005) Regulation of osteoblastogenesis and bone mass by Wnt10b. *Proc Natl Acad Sci USA* **102**: 3324-3329.

Bennett CN, Ouyang H, Ma YL, Zeng Q, Gerin I, Sousa KM, Lane TF, Krishnan V, Hankenson KD, MacDougald OA (2007) Wnt10b increases postnatal bone formation by enhancing osteoblast differentiation. *J Bone Miner Res* **22**: 1924-1932.

Berner A, Reichert JC, Muller MB, Zellner J, Pfeifer C, Dienstknecht T, Nerlich M, Sommerville S, Dickinson IC, Schutz MA, Fuchtmeyer B (2012) Treatment of long bone defects and non-unions: from research to clinical practice. *Cell Tissue Res* **347**: 501-519.

Betz OB, Betz VM, Nazarian A, Pilapil CG, Vrahas MS, Bouxsein ML, Gerstenfeld LC, Einhorn TA, Evans CH (2006) Direct percutaneous gene delivery to enhance healing of segmental bone defects. *J Bone Joint Surg Am* **88**: 355-365.

Betz OB, Betz VM, Nazarian A, Egermann M, Gerstenfeld LC, Einhorn TA, Vrahas MS, Bouxsein ML, Evans CH (2007a) Delayed administration of adenoviral BMP-2 vector improves the formation of bone in osseous defects. *Gene Ther* **14**: 1039-1044.

Betz VM, Betz OB, Glatt V, Gerstenfeld LC, Einhorn TA, Bouxsein ML, Vrahas MS, Evans CH (2007b) Healing of segmental bone defects by direct percutaneous gene delivery: effect of vector dose. *Hum Gene Ther* **18**: 907-915.

Bilic R, Simic P, Jelic M, Stern-Padovan R, Dodig D, van Meerdervoort HP, Martinovic S, Ivankovic D, Pecina M, Vukicevic S (2006) Osteogenic protein-1 (BMP-7) accelerates healing of scaphoid non-union with proximal pole sclerosis. *Int Orthop* **30**: 128-134.

Bland YS, Critchlow MA, Ashhurst DE (1995) Exogenous fibroblast growth factors-1 and -2 do not accelerate fracture healing in the rabbit. *Acta Orthop Scand* **66**: 543-548.

Blattert TR, Dellling G, Dalal PS, Toth CA, Balling H, Weckbach A (2002) Successful transpedicular lumbar interbody fusion by means of a composite of osteogenic protein-1 (rhBMP-7) and hydroxyapatite carrier: a comparison with autograft and hydroxyapatite in the sheep spine. *Spine* **27**: 2697-2705.

Blokhuis TJ, den Boer FC, Bramer JA, Jenner JM, Bakker FC, Patka P, Haarman HJ (2001) Biomechanical and histological aspects of fracture healing, stimulated with osteogenic protein-1. *Biomaterials* **22**: 725-730.

Blum JS, Barry MA, Mikos AG, Jansen JA (2003) *In vivo* evaluation of gene therapy vectors in *ex vivo*-derived marrow stromal cells for bone regeneration in a rat critical-size calvarial defect model. *Hum Gene Ther* **14**: 1689-1701.

Bodde EW, Boerman OC, Russel FG, Mikos AG, Spauwen PH, Jansen JA (2008) The kinetic and biological activity of different loaded rhBMP-2 calcium phosphate cement implants in rats. *J Biomed Mater Res A* **87**: 780-791.

Boden SD (2000) Biology of lumbar spine fusion and use of bone graft substitutes: present, future, and next generation. *Tissue Eng* **6**: 383-399.

Boerckel JD, Kolambkar YM, Stevens HY, Lin AS, Dupont KM, Guldberg RE (2012) Effects of *in vivo* mechanical loading on large bone defect regeneration. *J Orthop Res* **30**: 1067-1075.

Bonfield W (2006) Designing porous scaffolds for tissue engineering. *Philos Trans A Math Phys Eng Sci* **364**: 227-232.

Bordei P (2011) Locally applied platelet-derived growth factor accelerates fracture healing. *J Bone Joint Surg Br* **93**: 1653-1659.

Borgstrom F, Strom O, Coelho J, Johansson H, Oden A, McCloskey E, Kanis JA (2010a) The cost-effectiveness of strontium ranelate in the UK for the management of osteoporosis. *Osteoporos Int* **21**: 339-349.

Borgstrom F, Strom O, Coelho J, Johansson H, Oden A, McCloskey EV, Kanis JA (2010b) The cost-effectiveness of risedronate in the UK for the management of osteoporosis using the FRAX. *Osteoporos Int* **21**: 495-505.

Bostrom MP, Gamradt SC, Asnis P, Vickery BH, Hill E, Avnur Z, Waters RV (2000) Parathyroid hormone-related protein analog RS-66271 is an effective therapy for impaired bone healing in rabbits on corticosteroid therapy. *Bone* **26**: 437-442.

Bouxsein ML, Turek TJ, Blake CA, D'Augusta D, Li X, Stevens M, Seeherman HJ, Wozney JM (2001) Recombinant human bone morphogenetic protein-2 accelerates healing in a rabbit ulnar osteotomy model. *J Bone Joint Surg Am* **83**: 1219-1230.

Boyne PJ, Marx RE, Nevins M, Triplett G, Lazaro E, Lilly LC, Alder M, Nummikoski P (1997) A feasibility study evaluating rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. *Int J Periodontics Restorative Dent* **17**: 11-25.

Boyne PJ, Nath R, Nakamura A (1998) Human recombinant BMP-2 in osseous reconstruction of simulated cleft palate defects. *Br J Oral Maxillofac Surg* **36**: 84-90.

Boyne PJ, Lilly LC, Marx RE, Moy PK, Nevins M, Spagnoli DB, Triplett RG (2005) *De novo* bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. *J Oral Maxillofac Surg* **63**: 1693-1707.

Brick KE, Chen X, Lohr J, Schmidt AH, Kidder LS, Lew WD (2009) rhBMP-2 modulation of gene expression in infected segmental bone defects. *Clin Orthop Relat Res* **467**: 3096-3103.

Bright C, Park YS, Sieber AN, Kostuik JP, Leong KW (2006) *In vivo* evaluation of plasmid DNA encoding OP-1 protein for spine fusion. *Spine* **31**: 2163-2172.

Brouwers JE, van Rietbergen B, Huiskes R, Ito K (2009) Effects of PTH treatment on tibial bone of ovariectomized rats assessed by *in vivo* micro-CT. *Osteoporos Int* **20**: 1823-1835.

Brown KV, Li B, Guda T, Perrien DS, Guelcher SA, Wenke JC (2011) Improving bone formation in a rat femur segmental defect by controlling bone morphogenetic protein-2 release. *Tissue Eng Part A* **17**: 1735-1746.

- Burdick JA, Vunjak-Novakovic G (2009) Engineered microenvironments for controlled stem cell differentiation. *Tissue Eng Part A* **15**: 205-219.
- Burge RT, Worley D, Johanson A, Bhattacharyya S, Bose U (2001) The cost of osteoporotic fractures in the UK: projections for 2000-2020. *J Med Econ* **4**: 51-62.
- Burkhart KJ, Rommens PM (2008) Intramedullary application of bone morphogenetic protein in the management of a major bone defect after an Ilizarov procedure. *J Bone Joint Surg Br* **90**: 806-809.
- Burkus JK, Dorchak JD, Sanders DL (2003a) Radiographic assessment of interbody fusion using recombinant human bone morphogenetic protein type 2. *Spine* **28**: 372-377.
- Burkus JK, Heim SE, Gornet MF, Zdeblick TA (2003b) Is INFUSE bone graft superior to autograft bone? An integrated analysis of clinical trials using the LT-CAGE lumbar tapered fusion device. *J Spinal Disord Tech* **16**: 113-122.
- Burkus JK, Gornet MF, Schuler TC, Kleeman TJ, Zdeblick TA (2009) Six-year outcomes of anterior lumbar interbody arthrodesis with use of interbody fusion cages and recombinant human bone morphogenetic protein-2. *J Bone Joint Surg Am* **91**: 1181-1189.
- Butler DL, Goldstein SA, Guilak F (2000) Functional tissue engineering: the role of biomechanics. *J Biomech Eng* **122**: 570-575.
- Buttermann GR (2008) Prospective nonrandomized comparison of an allograft with bone morphogenic protein *versus* an iliac-crest autograft in anterior cervical discectomy and fusion. *Spine J* **8**: 426-435.
- Byrne AM, Bouchier-Hayes DJ, Harmey JH (2005) Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). *J Cell Mol Med* **9**: 777-794.
- Calori GM, Tagliabue L, Gala L, d'Imporzano M, Peretti G, Albisetti W (2008) Application of rhBMP-7 and platelet-rich plasma in the treatment of long bone non-unions: a prospective randomised clinical study on 120 patients. *Injury* **39**: 1391-1402.
- Campbell GM, Bernhardt R, Scharnweber D, Boyd SK (2011) The bone architecture is enhanced with combined PTH and alendronate treatment compared to monotherapy while maintaining the state of surface mineralization in the OVX rat. *Bone* **49**: 225-232.
- Carli A, Gao C, Khayat-Kholghi M, Li A, Wang H, Ladel C, Harvey EJ, Henderson JE (2012) FGF18 augments osseointegration of intra-medullary implants in osteopenic FGFR3(-/-) mice. *Eur Cell Mater* **24**: 107-116.
- Carreon LY, Glassman SD, Brock DC, Dimar JR, Puno RM, Campbell MJ (2008) Adverse events in patients re-exposed to bone morphogenetic protein for spine surgery. *Spine* **33**: 391-393.
- Carstens MH, Chin M, Li XJ (2005) *In situ* osteogenesis: regeneration of 10-cm mandibular defect in porcine model using recombinant human bone morphogenetic protein-2 (rhBMP-2) and Helistat absorbable collagen sponge. *J Craniofac Surg* **16**: 1033-1042.
- Carter TG, Brar PS, Tolas A, Beirne OR (2008) Off-label use of recombinant human bone morphogenetic protein-2 (rhBMP-2) for reconstruction of mandibular bone defects in humans. *J Oral Maxillofac Surg* **66**: 1417-1425.
- Cartmell S (2009) Controlled release scaffolds for bone tissue engineering. *J Pharm Sci* **98**: 430-441.
- Castaneda S, Largo R, Calvo E, Rodriguez-Salvanes F, Marcos ME, Diaz-Curiel M, Herrero-Beaumont G (2006) Bone mineral measurements of subchondral and trabecular bone in healthy and osteoporotic rabbits. *Skeletal Radiol* **35**: 34-41.
- Chan G, Mooney DJ (2008) New materials for tissue engineering: towards greater control over the biological response. *Trends Biotechnol* **26**: 382-392.
- Chang SC, Chuang HL, Chen YR, Chen JK, Chung HY, Lu YL, Lin HY, Tai CL, Lou J (2003) *Ex vivo* gene therapy in autologous bone marrow stromal stem cells for tissue-engineered maxillofacial bone regeneration. *Gene Ther* **10**: 2013-2019.
- Chang J, Sonoyama W, Wang Z, Jin Q, Zhang C, Krebsbach PH, Giannobile W, Shi S, Wang CY (2007) Noncanonical Wnt-4 signaling enhances bone regeneration of mesenchymal stem cells in craniofacial defects through activation of p38 MAPK. *J Biol Chem* **282**: 30938-30948.
- Chang PC, Cirelli JA, Jin Q, Seol YJ, Sugai JV, D'Silva NJ, Danciu TE, Chandler LA, Sosnowski BA, Giannobile WV (2009) Adenovirus encoding human platelet-derived growth factor-B delivered to alveolar bone defects exhibits safety and biodistribution profiles favorable for clinical use. *Hum Gene Ther* **20**: 486-496.
- Chang PC, Seol YJ, Cirelli JA, Pellegrini G, Jin Q, Franco LM, Goldstein SA, Chandler LA, Sosnowski B, Giannobile WV (2010) PDGF-B gene therapy accelerates bone engineering and oral implant osseointegration. *Gene Ther* **17**: 95-104.
- Chaofeng W, Chao Z, Deli W, Jianhong W, Yan Z, Cheng X, Hongkui X, Qing H, Dike R (2013) Nucleus pulposus cells expressing hBMP7 can prevent the degeneration of allogenic IVD in a canine transplantation model. *J Orthop Res* **31**: 1366-1373.
- Chen X, Kidder LS, Lew WD (2002) Osteogenic protein-1 induced bone formation in an infected segmental defect in the rat femur. *J Orthop Res* **20**: 142-150.
- Chen X, Schmidt AH, Tsukayama DT, Bourgeault CA, Lew WD (2006) Recombinant human osteogenic protein-1 induces bone formation in a chronically infected, internally stabilized segmental defect in the rat femur. *J Bone Joint Surg Am* **88**: 1510-1523.
- Chen Y, Whetstone HC, Youn A, Nadesan P, Chow EC, Lin AC, Alman BA (2007) Beta-catenin signaling pathway is crucial for bone morphogenetic protein 2 to induce new bone formation. *J Biol Chem* **282**: 526-533.
- Choi SJ, Na K, Kim S, Woo DG, Sun BK, Chung HM, Park KH (2007) Combination of ascorbate and growth factor (TGF beta-3) in thermo-reversible hydrogel constructs embedded with rabbit chondrocytes for neocartilage formation. *J Biomed Mater Res A* **83**: 897-905.
- Choi KS, Ahn SY, Kim TS, Kim J, Kim BG, Han KH, Ban SJ, Kim HS, Choi Y, Lim CJ (2011) Characterization and biodistribution of human mesenchymal stem cells transduced with lentiviral-mediated BMP2. *Arch Pharm Res* **34**: 599-606.

- Chu TM, Warden SJ, Turner CH, Stewart RL (2007) Segmental bone regeneration using a load-bearing biodegradable carrier of bone morphogenetic protein-2. *Biomaterials* **28**: 459-467.
- Chung YI, Ahn KM, Jeon SH, Lee SY, Lee JH, Tae G (2007) Enhanced bone regeneration with BMP-2 loaded functional nanoparticle-hydrogel complex. *J Control Release* **121**: 91-99.
- Cicciu M, Herford AS, Stoffella E, Cervino G, Cicciu D (2012) Protein-signaled guided bone regeneration using titanium mesh and Rh-BMP2 in oral surgery: A case report involving left mandibular reconstruction after tumor resection. *Open Dent J* **6**: 51-55.
- Cipitria A, Reichert JC, Epari DR, Saifzadeh S, Berner A, Schell H, Mehta M, Schuetz MA, Duda GN, Hutmacher DW (2013) Polycaprolactone scaffold and reduced rhBMP-7 dose for the regeneration of critical-sized defects in sheep tibiae. *Biomaterials* **34**: 9960-9968.
- Cohen S, Bano MC, Cima LG, Allcock HR, Vacanti JP, Vacanti CA, Langer R (1993) Design of synthetic polymeric structures for cell transplantation and tissue engineering. *Clin Mater* **13**: 3-10.
- Cook SD, Baffes GC, Wolfe MW, Sampath TK, Rueger DC (1994a) Recombinant human bone morphogenetic protein-7 induces healing in a canine long-bone segmental defect model. *Clin Orthop Relat Res* **301**: 302-312.
- Cook SD, Baffes GC, Wolfe MW, Sampath TK, Rueger DC, Whitecloud TS, 3rd (1994b) The effect of recombinant human osteogenic protein-1 on healing of large segmental bone defects. *J Bone Joint Surg Am* **76**: 827-838.
- Cook SD, Wolfe MW, Salkeld SL, Rueger DC (1995) Effect of recombinant human osteogenic protein-1 on healing of segmental defects in non-human primates. *J Bone Joint Surg Am* **77**: 734-750.
- Cook SD, Salkeld SL, Brinker MR, Wolfe MW, Rueger DC (1998) Use of an osteoinductive biomaterial (rhOP-1) in healing large segmental bone defects. *J Orthop Trauma* **12**: 407-412.
- Cook SD, Salkeld SL, Patron LP, Sargent MC, Rueger DC (2002) Healing course of primate ulna segmental defects treated with osteogenic protein-1. *J Invest Surg* **15**: 69-79.
- Cook SD, Salkeld SL, Patron LP (2005) Bone defect healing with an osteogenic protein-1 device combined with carboxymethylcellulose. *J Biomed Mater Res B Appl Biomater* **75**: 137-145.
- Cooper C, Atkinson EJ, O'Fallon WM, Melton LJ, 3rd (1992a) Incidence of clinically diagnosed vertebral fractures: a population-based study in Rochester, Minnesota, 1985-1989. *J Bone Miner Res* **7**: 221-227.
- Cooper C, Campion G, Melton LJ, 3rd (1992b) Hip fractures in the elderly: a world-wide projection. *Osteoporos Int* **2**: 285-289.
- Cooper GM, Mooney MP, Gosain AK, Campbell PG, Losee JE, Huard J (2010) Testing the critical size in calvarial bone defects: revisiting the concept of a critical-size defect. *Plast Reconstr Surg* **125**: 1685-1692.
- Cornish J, Callon KE, Nicholson GC, Reid IR (1997) Parathyroid hormone-related protein-(107-139) inhibits bone resorption *in vivo*. *Endocrinology* **138**: 1299-1304.
- Cowan CM, Aghaloo T, Chou YF, Walder B, Zhang X, Soo C, Ting K, Wu B (2007) MicroCT evaluation of three-dimensional mineralization in response to BMP-2 doses *in vitro* and in critical sized rat calvarial defects. *Tissue Eng* **13**: 501-512.
- Cullinane DM, Lietman SA, Inoue N, Deitz LW, Chao EY (2002) The effect of recombinant human osteogenic protein-1 (bone morphogenetic protein-7) impregnation on allografts in a canine intercalary bone defect. *J Orthop Res* **20**: 1240-1245.
- Dahabreh Z, Dimitriou R, Giannoudis PV (2007) Health economics: a cost analysis of treatment of persistent fracture non-unions using bone morphogenetic protein-7. *Injury* **38**: 371-377.
- Daugaard H, Elmengaard B, Andreassen T, Bechtold J, Lamberg A, Soballe K (2011) Parathyroid hormone treatment increases fixation of orthopedic implants with gap healing: a biomechanical and histomorphometric canine study of porous coated titanium alloy implants in cancellous bone. *Calcif Tissue Int* **88**: 294-303.
- Daugaard H, Elmengaard B, Andreassen TT, Lamberg A, Bechtold JE, Soballe K (2012) Systemic intermittent parathyroid hormone treatment improves osseointegration of press-fit inserted implants in cancellous bone. *Acta Orthop* **83**: 411-419.
- Dawson E, Bae HW, Burkus JK, Stambough JL, Glassman SD (2009) Recombinant human bone morphogenetic protein-2 on an absorbable collagen sponge with an osteoconductive bulking agent in posterolateral arthrodesis with instrumentation. A prospective randomized trial. *J Bone Joint Surg Am* **91**: 1604-1613.
- Dawson JJ, Kanczler J, Tare R, Kassem M, Oreffo RO (2014) Concise review: bridging the gap: bone regeneration using skeletal stem cell-based strategies - where are we now? *Stem Cells* **32**: 35-44.
- De Boer J, Wang HJ, Van Blitterswijk C (2004) Effects of Wnt signaling on proliferation and differentiation of human mesenchymal stem cells. *Tissue Eng* **10**: 393-401.
- de Castro LF, Lozano D, Portal-Nunez S, Maycas M, De la Fuente M, Caeiro JR, Esbrit P (2011) Comparison of the skeletal effects induced by daily administration of PTHrP (1-36) and PTHrP (107-139) to ovariectomized mice. *J Cell Physiol* **227**: 1752-1760.
- Delgado JJ, Sanchez E, Baro M, Reyes R, Evora C, Delgado A (2012) A platelet derived growth factor delivery system for bone regeneration. *J Mater Sci Mater Med* **23**: 1903-1912.
- Delmas PD, Vergnaud P, Arlot ME, Pastoureaux P, Meunier PJ, Nilsson MH (1995) The anabolic effect of human PTH (1-34) on bone formation is blunted when bone resorption is inhibited by the bisphosphonate tiludronate--is activated resorption a prerequisite for the *in vivo* effect of PTH on formation in a remodeling system? *Bone* **16**: 603-610.
- Delmas PD, van de Langerijt L, Watts NB, Eastell R, Genant H, Grauer A, Cahall DL (2005) Underdiagnosis of vertebral fractures is a worldwide problem: the IMPACT study. *J Bone Miner Res* **20**: 557-563.
- den Boer FC, Bramer JA, Blokhuis TJ, Van Soest EJ, Jenner JM, Patka P, Bakker FC, Burger EH, Haarman HJ

- (2002) Effect of recombinant human osteogenic protein-1 on the healing of a freshly closed diaphyseal fracture. *Bone* **31**: 158-164.
- den Boer FC, Wippermann BW, Blokhuis TJ, Patka P, Bakker FC, Haarman HJ (2003) Healing of segmental bone defects with granular porous hydroxyapatite augmented with recombinant human osteogenic protein-1 or autologous bone marrow. *J Orthop Res* **21**: 521-528.
- Dickinson BP, Ashley RK, Wasson KL, O'Hara C, Gabbay J, Heller JB, Bradley JP (2008) Reduced morbidity and improved healing with bone morphogenetic protein-2 in older patients with alveolar cleft defects. *Plast Reconstr Surg* **121**: 209-217.
- Dimar JR, 2nd, Glassman SD, Burkus JK, Pryor PW, Hardacker JW, Carreon LY (2009) Clinical and radiographic analysis of an optimized rhBMP-2 formulation as an autograft replacement in posterolateral lumbar spine arthrodesis. *J Bone Joint Surg Am* **91**: 1377-1386.
- Dimitriou R, Dahabreh Z, Katsoulis E, Matthews SJ, Branfoot T, Giannoudis PV (2005) Application of recombinant BMP-7 on persistent upper and lower limb non-unions. *Injury* **36 Suppl 4**: S51-59.
- Djapic T, Kusec V, Jelic M, Vukicevic S, Pecina M (2003) Compressed homologous cancellous bone and bone morphogenetic protein (BMP)-7 or bone marrow accelerate healing of long-bone critical defects. *Int Orthop* **27**: 326-330.
- Dohin B, Dahan-Oliel N, Fassier F, Hamdy R (2009) Enhancement of difficult nonunion in children with osteogenic protein-1 (OP-1): early experience. *Clin Orthop Relat Res* **467**: 3230-3238.
- Dohzono S, Imai Y, Nakamura H, Wakitani S, Takaoka K (2009) Successful spinal fusion by E. coli-derived BMP-2-adsorbed porous beta-TCP granules: a pilot study. *Clin Orthop Relat Res* **467**: 3206-3212.
- Doi Y, Miyazaki M, Yoshiiwa T, Hara K, Kataoka M, Tsumura H (2011) Manipulation of the anabolic and catabolic responses with BMP-2 and zoledronic acid in a rat femoral fracture model. *Bone* **49**: 777-782.
- Donaldson LJ, Reckless IP, Scholes S, Mindell JS, Shelton NJ (2008) The epidemiology of fractures in England. *J Epidemiol Community Health* **62**: 174-180.
- Donati D, Di Bella C, Lucarelli E, Dozza B, Frisoni T, Aldini NN, Giardino R (2008) OP-1 application in bone allograft integration: preliminary results in sheep experimental surgery. *Injury* **39 Suppl 2**: S65-72.
- Duneas N, Crooks J, Ripamonti U (1998) Transforming growth factor-beta 1: induction of bone morphogenetic protein genes expression during endochondral bone formation in the baboon, and synergistic interaction with osteogenic protein-1 (BMP-7). *Growth Factors* **15**: 259-277.
- Dunn CA, Jin Q, Taba M, Jr, Franceschi RT, Bruce Rutherford R, Giannobile WV (2005) BMP gene delivery for alveolar bone engineering at dental implant defects. *Mol Ther* **11**: 294-299.
- Dunstan CR, Boyce R, Boyce BF, Garrett IR, Izbicka E, Burgess WH, Mundy GR (1999) Systemic administration of acidic fibroblast growth factor (FGF-1) prevents bone loss and increases new bone formation in ovariectomized rats. *J Bone Miner Res* **14**: 953-959.
- Dupont KM, Boerckel JD, Stevens HY, Diab T, Kolambkar YM, Takahata M, Schwarz EM, Guldberg RE (2012) Synthetic scaffold coating with adeno-associated virus encoding BMP2 to promote endogenous bone repair. *Cell Tissue Res* **347**: 575-588.
- Edwards RB, 3rd, Seeherman HJ, Bogdanske JJ, Devitt J, Vanderby R, Jr, Markel MD (2004) Percutaneous injection of recombinant human bone morphogenetic protein-2 in a calcium phosphate paste accelerates healing of a canine tibial osteotomy. *J Bone Joint Surg Am* **86**: 1425-1438.
- Egermann M, Goldhahn J, Schneider E (2005) Animal models for fracture treatment in osteoporosis. *Osteoporos Int* **16 Suppl 2**: S129-138.
- Egermann M, Lill CA, Griesbeck K, Evans CH, Robbins PD, Schneider E, Baltzer AW (2006) Effect of BMP-2 gene transfer on bone healing in sheep. *Gene Ther* **13**: 1290-1299.
- Ekrol I, Hajducka C, Court-Brown C, McQueen MM (2008) A comparison of RhBMP-7 (OP-1) and autogenous graft for metaphyseal defects after osteotomy of the distal radius. *Injury* **39 Suppl 2**: S73-82.
- El Backly RM, Zaky SH, Muraglia A, Tonachini L, Brun F, Canciani B, Chiapale D, Santolini F, Cancedda R, Mastrogiacomo M (2013) A platelet-rich plasma-based membrane as a periosteal substitute with enhanced osteogenic and angiogenic properties: a new concept for bone repair. *Tissue Eng Part A* **19**: 152-165.
- El Haj AJ, Wood MA, Thomas P, Yang Y (2005) Controlling cell biomechanics in orthopaedic tissue engineering and repair. *Pathol Biol* **53**: 581-589.
- Engel E, Del Valle S, Aparicio C, Altankov G, Asin L, Planell JA, Ginebra MP (2008) Discerning the role of topography and ion exchange in cell response of bioactive tissue engineering scaffolds. *Tissue Eng Part A* **14**: 1341-1351.
- Even J, Eskander M, Kang J (2012) Bone morphogenetic protein in spine surgery: current and future uses. *J Am Acad Orthop Surg* **20**: 547-552.
- Fabeck L, Ghafil D, Gerroudj M, Baillon R, Delince P (2006) Bone morphogenetic protein 7 in the treatment of congenital pseudarthrosis of the tibia. *J Bone Joint Surg Br* **88**: 116-118.
- Fiorellini JP, Howell TH, Cochran D, Malmquist J, Lilly LC, Spagnoli D, Toljanic J, Jones A, Nevins M (2005) Randomized study evaluating recombinant human bone morphogenetic protein-2 for extraction socket augmentation. *J Periodontol* **76**: 605-613.
- Forriol F, Longo UG, Concejo C, Ripalda P, Maffulli N, Denaro V (2009) Platelet-rich plasma, rhOP-1 (rhBMP-7) and frozen rib allograft for the reconstruction of bony mandibular defects in sheep. A pilot experimental study. *Injury* **40 Suppl 3**: S44-49.
- Fraher LJ, Hodsman AB, Jonas K, Saunders D, Rose CI, Henderson JE, Hendy GN, Goltzman D (1992) A comparison of the *in vivo* biochemical responses to exogenous parathyroid hormone-(1-34) [PTH-(1-34)] and PTH-related peptide-(1-34) in man. *J Clin Endocrinol Metab* **75**: 417-423.
- Friedlaender GE, Perry CR, Cole JD, Cook SD, Cierny G, Muschler GF, Zych GA, Calhoun JH, LaForte AJ,

- Yin S (2001) Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. *J Bone Joint Surg Am* **83 Suppl 1**: S151-158.
- Fu YC, Nie H, Ho ML, Wang CK, Wang CH (2008) Optimized bone regeneration based on sustained release from three-dimensional fibrous PLGA/HAP composite scaffolds loaded with BMP-2. *Biotechnol Bioeng* **99**: 996-1006.
- Fu TS, Chen WJ, Chen LH, Lin SS, Liu SJ, Ueng SW (2009) Enhancement of posterolateral lumbar spine fusion using low-dose rhBMP-2 and cultured marrow stromal cells. *J Orthop Res* **27**: 380-384.
- Fu K, Xu Q, Czernuszka J, McKenna CE, Ebetino FH, Russell RG, Triffitt JT, Xia Z (2010) Prolonged osteogenesis from human mesenchymal stem cells implanted in immunodeficient mice by using coralline hydroxyapatite incorporating rhBMP2 microspheres. *J Biomed Mater Res A* **92**: 1256-1264.
- Fu R, Selph S, McDonagh M, Peterson K, Tiwari A, Chou R, Helfand M (2013) Effectiveness and harms of recombinant human bone morphogenetic protein-2 in spine fusion: a systematic review and meta-analysis. *Ann Intern Med* **158**: 890-902.
- Fujimura K, Bessho K, Okubo Y, Kusumoto K, Segami N, Iizuka T (2002) The effect of fibroblast growth factor-2 on the osteoinductive activity of recombinant human bone morphogenetic protein-2 in rat muscle. *Arch Oral Biol* **47**: 577-584.
- Fukuroku J, Inoue N, Rafiee B, Sim FH, Frassica FJ, Chao EY (2007) Extracortical bone-bridging fixation with use of cortical allograft and recombinant human osteogenic protein-1. *J Bone Joint Surg Am* **89**: 1486-1496.
- Gao Y, Zhu S, Luo E, Li J, Feng G, Hu J (2009) Basic fibroblast growth factor suspended in Matrigel improves titanium implant fixation in ovariectomized rats. *J Control Release* **139**: 15-21.
- Gazit D, Turgeman G, Kelley P, Wang E, Jalenak M, Zilberman Y, Moutsatsos I (1999) Engineered pluripotent mesenchymal cells integrate and differentiate in regenerating bone: a novel cell-mediated gene therapy. *J Gene Med* **1**: 121-133.
- Geesink RG, Hoefnagels NH, Bulstra SK (1999) Osteogenic activity of OP-1 bone morphogenetic protein (BMP-7) in a human fibular defect. *J Bone Joint Surg Br* **81**: 710-718.
- Geetha-Loganathan P, Nimmagadda S, Christ B, Huang R, Scaal M (2010) Ectodermal Wnt6 is an early negative regulator of limb chondrogenesis in the chicken embryo. *BMC Dev Biol* **10**: 32.
- Geiger F, Lorenz H, Xu W, Szalay K, Kasten P, Claes L, Augat P, Richter W (2007) VEGF producing bone marrow stromal cells (BMSC) enhance vascularization and resorption of a natural coral bone substitute. *Bone* **41**: 516-522.
- Gelse K, Klinger P, Koch M, Surmann-Schmitt C, von der Mark K, Swoboda B, Hennig FF, Gusinde J (2011) Thrombospondin-1 prevents excessive ossification in cartilage repair tissue induced by osteogenic protein-1. *Tissue Eng Part A* **17**: 2101-2112.
- Gerhart TN, Kirker-Head CA, Kriz MJ, Holtrop ME, Hennig GE, Hipp J, Schelling SH, Wang E (1993) Healing segmental femoral defects in sheep using recombinant human bone morphogenetic protein. *Clin Orthop Relat Res* **293**: 317-326.
- Geuze RE, Theyse LF, Kempen DH, Hazewinkel HA, Kraak HY, Oner FC, Dhert WJ, Alblas J (2012) A differential effect of bone morphogenetic protein-2 and vascular endothelial growth factor release timing on osteogenesis at ectopic and orthotopic sites in a large-animal model. *Tissue Eng Part A* **18**: 2052-2062.
- Ghosh K, Ingber DE (2007) Micromechanical control of cell and tissue development: implications for tissue engineering. *Adv Drug Deliv Rev* **59**: 1306-1318.
- Giannoudis PV, Psarakis S, Kanakaris NK, Pape HC (2007) Biological enhancement of bone healing with bone morphogenetic protein-7 at the clinical setting of pelvic girdle non-unions. *Injury* **38 Suppl 4**: S43-48.
- Giannoudis PV, Kanakaris NK, Dimitriou R, Gill I, Kolimarala V, Montgomery RJ (2009) The synergistic effect of autograft and BMP-7 in the treatment of atrophic nonunions. *Clin Orthop Relat Res* **467**: 3239-3248.
- Glassman SD, Carreon L, Djurasovic M, Campbell MJ, Puno RM, Johnson JR, Dimar JR (2007) Posterolateral lumbar spine fusion with INFUSE bone graft. *Spine J* **7**: 44-49.
- Glatt V, Kwong FN, Park K, Parry N, Griffin D, Vrahas M, Evans CH, Harris M (2009) Ability of recombinant human bone morphogenetic protein 2 to enhance bone healing in the presence of tobramycin: evaluation in a rat segmental defect model. *J Orthop Trauma* **23**: 693-701.
- Gomes PS, Fernandes MH (2010) Rodent models in bone-related research: the relevance of calvarial defects in the assessment of bone regeneration strategies. *Lab Anim* **45**: 14-24.
- Goodman SB, Song Y, Yoo JY, Fox N, Trindade MC, Kajiyama G, Ma T, Regula D, Brown J, Smith RL (2003) Local infusion of FGF-2 enhances bone ingrowth in rabbit chambers in the presence of polyethylene particles. *J Biomed Mater Res A* **65**: 454-461.
- Govender S, Csimma C, Genant HK, Valentin-Opran A, Amit Y, Arbel R, Aro H, Atar D, Bishay M, Borner MG, Chiron P, Choong P, Cinats J, Courtenay B, Feibel R, Geulette B, Gravel C, Haas N, Raschke M, Hammacher E, van der Velde D, Hardy P, Holt M, Josten C, Ketterl RL, Lindeque B, Lob G, Mathevon H, McCoy G, Marsh D, Miller R, Munting E, Oevre S, Nordsletten L, Patel A, Pohl A, Rennie W, Reynders P, Rommens PM, Rondia J, Rossouw WC, Daneel PJ, Ruff S, Ruter A, Santavirta S, Schildhauer TA, Gekle C, Schnettler R, Segal D, Seiler H, Snowdowne RB, Stapert J, Taglang G, Verdonk R, Vogels L, Weckbach A, Wentzensen A, Wisniewski T (2002) Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. *J Bone Joint Surg Am* **84**: 2123-2134.
- Grauer JN, Vaccaro AR, Kato M, Kwon BK, Beiner JM, Patel TC, Hilibrand AS, Chiba K, Albert TJ (2004) Development of a New Zealand white rabbit model of spinal pseudarthrosis repair and evaluation of the potential role of OP-1 to overcome pseudarthrosis. *Spine* **29**: 1405-1412.

Gray SK, McGee-Lawrence ME, Sanders JL, Condon KW, Tsai CJ, Donahue SW (2012) Black bear parathyroid hormone has greater anabolic effects on trabecular bone in dystrophin-deficient mice than in wild type mice. *Bone* **51**: 578-585.

Green D, Walsh D, Mann S, Oreffo RO (2002) The potential of biomimesis in bone tissue engineering: lessons from the design and synthesis of invertebrate skeletons. *Bone* **30**: 810-815.

Grimes R, Jepsen KJ, Fitch JL, Einhorn TA, Gerstenfeld LC (2011) The transcriptome of fracture healing defines mechanisms of coordination of skeletal and vascular development during endochondral bone formation. *J Bone Miner Res* **26**: 2597-2609.

Groeneveld EH, van den Bergh JP, Holzmann P, ten Bruggenkate CM, Tuinzing DB, Burger EH (1999) Histomorphometrical analysis of bone formed in human maxillary sinus floor elevations grafted with OP-1 device, demineralized bone matrix or autogenous bone. Comparison with non-grafted sites in a series of case reports. *Clin Oral Implants Res* **10**: 499-509.

Gu Y, Chen L, Yang HL, Luo ZP, Tang TS (2011) Evaluation of an injectable silk fibroin enhanced calcium phosphate cement loaded with human recombinant bone morphogenetic protein-2 in ovine lumbar interbody fusion. *J Biomed Mater Res A* **97**: 177-185.

Guan L, Davies JE (2004) Preparation and characterization of a highly macroporous biodegradable composite tissue engineering scaffold. *J Biomed Mater Res A* **71**: 480-487.

Gugala Z, Lindsey RW, Gogolewski S (2007) New approaches in the treatment of critical-size segmental defects in long bones. *Macro Symp* **253**: 147-161.

Gullberg B, Johnell O, Kanis JA (1997) World-wide projections for hip fracture. *Osteoporos Int* **7**: 407-413.

Guo X, Zheng Q, Kulbatski I, Yuan Q, Yang S, Shao Z, Wang H, Xiao B, Pan Z, Tang S (2006) Bone regeneration with active angiogenesis by basic fibroblast growth factor gene transfected mesenchymal stem cells seeded on porous beta-TCP ceramic scaffolds. *Biomed Mater* **1**: 93-99.

Gutwald R, Haberstroh J, Stricker A, Ruther E, Otto F, Xavier SP, Oshima T, Marukawa E, Seto I, Enomoto S, Hoogendijk CF, Schmelzeisen R, Sauerbier S (2010) Influence of rhBMP-2 on bone formation and osseointegration in different implant systems after sinus-floor elevation. An *in vivo* study on sheep. *J Craniomaxillofac Surg* **38**: 571-579.

Haid RW, Jr, Branch CL, Jr, Alexander JT, Burkus JK (2004) Posterior lumbar interbody fusion using recombinant human bone morphogenetic protein type 2 with cylindrical interbody cages. *Spine J* **4**: 527-538.

Haidar ZS, Hamdy RC, Tabrizian M (2010a) Biocompatibility and safety of a hybrid core-shell nanoparticulate OP-1 delivery system intramuscularly administered in rats. *Biomaterials* **31**: 2746-2754.

Haidar ZS, Tabrizian M, Hamdy RC (2010b) A hybrid rhOP-1 delivery system enhances new bone regeneration and consolidation in a rabbit model of distraction osteogenesis. *Growth Factors* **28**: 44-55.

Hak DJ, Makino T, Niikura T, Hazelwood SJ, Curtiss S, Reddi AH (2006) Recombinant human BMP-7 effectively

prevents non-union in both young and old rats. *J Orthop Res* **24**: 11-20.

Hall SL, Lau KH, Chen ST, Wergedal JE, Srivastava A, Klamut H, Sheng MH, Gridley DS, Mohan S, Baylink DJ (2007) Sca-1(+) hematopoietic cell-based gene therapy with a modified FGF-2 increased endosteal/trabecular bone formation in mice. *Mol Ther* **15**: 1881-1889.

Hamdy RC, Amako M, Beckman L, Kawaguchi M, Rauch F, Lauzier D, Steffen T (2003) Effects of osteogenic protein-1 on distraction osteogenesis in rabbits. *Bone* **33**: 248-255.

Han SH, Kim YH, Park MS, Kim IA, Shin JW, Yang WI, Jee KS, Park KD, Ryu GH, Lee JW (2008) Histological and biomechanical properties of regenerated articular cartilage using chondrogenic bone marrow stromal cells with a PLGA scaffold *in vivo*. *J Biomed Mater Res A* **87**: 850-861.

Hasharoni A, Zilberman Y, Turgeman G, Helm GA, Liebergall M, Gazit D (2005) Murine spinal fusion induced by engineered mesenchymal stem cells that conditionally express bone morphogenetic protein-2. *J Neurosurg Spine* **3**: 47-52.

Hayashi C, Hasegawa U, Saita Y, Hemmi H, Hayata T, Nakashima K, Ezura Y, Amagasa T, Akiyoshi K, Noda M (2009) Osteoblastic bone formation is induced by using nanogel-crosslinking hydrogel as novel scaffold for bone growth factor. *J Cell Physiol* **220**: 1-7.

He D, Genecov DG, Herbert M, Barcelo R, Elsalanty ME, Weprin BE, Opperman LA (2009) Effect of recombinant human bone morphogenetic protein-2 on bone regeneration in large defects of the growing canine skull after dura mater replacement with a dura mater substitute. *J Neurosurg* **112**: 319-328.

Heckman JD, Boyan BD, Aufdemorte TB, Abbott JT (1991) The use of bone morphogenetic protein in the treatment of non-union in a canine model. *J Bone Joint Surg Am* **73**: 750-764.

Henslee AM, Spicer PP, Yoon DM, Nair MB, Meretoja VV, Witherel KE, Jansen JA, Mikos AG, Kasper FK (2011) Biodegradable composite scaffolds incorporating an intramedullary rod and delivering bone morphogenetic protein-2 for stabilization and bone regeneration in segmental long bone defects. *Acta Biomater* **7**: 3627-3637.

Herford AS, Boyne PJ (2008) Reconstruction of mandibular continuity defects with bone morphogenetic protein-2 (rhBMP-2). *J Oral Maxillofac Surg* **66**: 616-624.

Herford AS, Cicciu M (2012) Bone resorption analysis of platelet-derived growth factor type BB application on collagen for bone grafts secured by titanium mesh over a pig jaw defect model. *Natl J Maxillofac Surg* **3**: 172-179.

Herford AS, Lu M, Akin L, Cicciu M (2012) Evaluation of a porcine matrix with and without platelet-derived growth factor for bone graft coverage in pigs. *Int J Oral Maxillofac Implants* **27**: 1351-1358.

Hernandez A, Reyes R, Sanchez E, Rodriguez-Evora M, Delgado A, Evora C (2012) *In vivo* osteogenic response to different ratios of BMP-2 and VEGF released from a biodegradable porous system. *J Biomed Mater Res A* **100**: 2382-2391.

Hernandez-Alfaro F, Ruiz-Magaz V, Chatakun P, Guijarro-Martinez R (2012) Mandibular reconstruction with

tissue engineering in multiple recurrent ameloblastoma. *Int J Periodontics Restorative Dent* **32**: e82-86.

Hidaka C, Goshi K, Rawlins B, Boachie-Adjei O, Crystal RG (2003) Enhancement of spine fusion using combined gene therapy and tissue engineering BMP-7-expressing bone marrow cells and allograft bone. *Spine* **28**: 2049-2057.

Higuchi T, Kinoshita A, Takahashi K, Oda S, Ishikawa I (1999) Bone regeneration by recombinant human bone morphogenetic protein-2 in rat mandibular defects. An experimental model of defect filling. *J Periodontol* **70**: 1026-1031.

Hirata E, Menard-Moyon C, Venturelli E, Takita H, Watari F, Bianco A, Yokoyama A (2013) Carbon nanotubes functionalized with fibroblast growth factor accelerate proliferation of bone marrow-derived stromal cells and bone formation. *Nanotechnology* **24**: 435101.

Hock JM, Fonseca J, Gunness-Hey M, Kemp BE, Martin TJ (1989) Comparison of the anabolic effects of synthetic parathyroid hormone-related protein (PTHrP) 1-34 and PTH 1-34 on bone in rats. *Endocrinology* **125**: 2022-2027.

Hock JM, Gera I (1992) Effects of continuous and intermittent administration and inhibition of resorption on the anabolic response of bone to parathyroid hormone. *J Bone Miner Res* **7**: 65-72.

Hong KS, Kim EC, Bang SH, Chung CH, Lee YI, Hyun JK, Lee HH, Jang JH, Kim TI, Kim HW (2010) Bone regeneration by bioactive hybrid membrane containing FGF2 within rat calvarium. *J Biomed Mater Res A* **94**: 1187-1194.

Horner EA, Kirkham J, Wood D, Curran S, Smith M, Thomson B, Yang XB (2010) Long bone defect models for tissue engineering applications: criteria for choice. *Tissue Eng Part B Rev* **16**: 263-271.

Horwitz MJ, Tedesco MB, Sereika SM, Syed MA, Garcia-Ocana A, Bisello A, Hollis BW, Rosen CJ, Wysolmerski JJ, Dann P, Gundberg C, Stewart AF (2005) Continuous PTH and PTHrP infusion causes suppression of bone formation and discordant effects on 1,25(OH)₂ vitamin D. *J Bone Miner Res* **20**: 1792-1803.

Horwitz MJ, Tedesco MB, Sereika SM, Prebehala L, Gundberg CM, Hollis BW, Bisello A, Garcia-Ocana A, Carneiro RM, Stewart AF (2011) A 7-day continuous infusion of PTH or PTHrP suppresses bone formation and uncouples bone turnover. *J Bone Miner Res* **26**: 2287-2297.

Hosokawa R, Kikuzaki K, Kimoto T, Matsuura T, Chiba D, Wadamoto M, Sato Y, Maeda M, Sano A, Akagawa Y (2000) Controlled local application of basic fibroblast growth factor (FGF-2) accelerates the healing of GBR. An experimental study in beagle dogs. *Clin Oral Implants Res* **11**: 345-353.

Hou R, Chen F, Yang Y, Cheng X, Gao Z, Yang HO, Wu W, Mao T (2007) Comparative study between coral-mesenchymal stem cells-rhBMP-2 composite and auto-bone-graft in rabbit critical-sized cranial defect model. *J Biomed Mater Res A* **80**: 85-93.

Hou J, Wang J, Cao L, Qian X, Xing W, Lu J, Liu C (2012) Segmental bone regeneration using rhBMP-2-loaded collagen/chitosan microspheres composite scaffold in a rabbit model. *Biomed Mater* **7**: 035002.

Howard D, BATTERY LD, Shakesheff KM, Roberts SJ (2008) Tissue engineering: strategies, stem cells and scaffolds. *J Anat* **213**: 66-72.

Hussein KA, Zakhary IE, Elawady AR, Emam HA, Sharawy M, Baban B, Akeel S, Al-Shabrawey M, Elsalanty ME (2012) Difference in soft tissue response between immediate and delayed delivery suggests a new mechanism for recombinant human bone morphogenetic protein 2 action in large segmental bone defects. *Tissue Eng Part A* **18**: 665-675.

Hwang CJ, Vaccaro AR, Hong J, Lawrence JP, Fischgrund JS, Alaoui-Ismaili MH, Falb D (2010) Immunogenicity of osteogenic protein 1: results from a prospective, randomized, controlled, multicenter pivotal study of uninstrumented lumbar posterolateral fusion. *J Neurosurg Spine* **13**: 484-493.

Hyun SJ, Han DK, Choi SH, Chai JK, Cho KS, Kim CK, Kim CS (2005) Effect of recombinant human bone morphogenetic protein-2, -4, and -7 on bone formation in rat calvarial defects. *J Periodontol* **76**: 1667-1674.

Inui K, Maeda M, Sano A, Fujioka K, Yutani Y, Sakawa A, Yamano Y, Kato Y, Koike T (1998) Local application of basic fibroblast growth factor minipellet induces the healing of segmental bony defects in rabbits. *Calcif Tissue Int* **63**: 490-495.

Ishihara A, Shields KM, Litsky AS, Mattoon JS, Weisbrode SE, Bartlett JS, Bertone AL (2008) Osteogenic gene regulation and relative acceleration of healing by adenoviral-mediated transfer of human BMP-2 or -6 in equine osteotomy and ostectomy models. *J Orthop Res* **26**: 764-771.

Ishihara A, Zekas LJ, Litsky AS, Weisbrode SE, Bertone AL (2010) Dermal fibroblast-mediated BMP2 therapy to accelerate bone healing in an equine osteotomy model. *J Orthop Res* **28**: 403-411.

Issa JP, Pitol DL, Iyomasa MM, Barbosa AP, Defino HL, Volpon JB, Shimano AC, Silva P (2009) Collagen fibers evaluation after rhBMP-2 insertion in critical-sized defects. *Micron* **40**: 560-562.

Issa JP, Defino HL, Netto JC, Volpon JB, Regalo SC, Iyomasa MM, Siessere S, Tiossi R (2010) Evaluation of rhBMP-2 and natural latex as potential osteogenic proteins in critical size defects by histomorphometric methods. *Anat Rec* **293**: 794-801.

Issa JP, Defino HL, Pereira YC, Netto JC, Sebald W, Bentley MV, Iyomasa MM, Ervolino E (2012) Bone repair investigation using rhBMP-2 and angiogenic protein extracted from latex. *Microsc Res Tech* **75**: 145-152.

Itoh T, Mochizuki M, Nishimura R, Matsunaga S, Kadosawa T, Kokubo S, Yokota S, Sasaki N (1998) Repair of ulnar segmental defect by recombinant human bone morphogenetic protein-2 in dogs. *J Vet Med Sci* **60**: 451-458.

Iwaniec UT, Magee KA, Mitova-Caneva NG, Wronski TJ (2003) Bone anabolic effects of subcutaneous treatment with basic fibroblast growth factor alone and in combination with estrogen in osteopenic ovariectomized rats. *Bone* **33**: 380-386.

Iyomasa MM, Issa JP, de Queiroz Tavares ML, Pereira YC, Stuaní MB, Mishima F, Coutinho-Netto J, Sebald W (2012) Influence of low-level laser associated with

osteogenic proteins recombinant human BMP-2 and Hevea brasiliensis on bone repair in Wistar rats. *Microsc Res Tech* **75**: 117-125.

Jacobson JA, Yanoso-Scholl L, Reynolds DG, Dadali T, Bradica G, Bukata S, Puzas EJ, Zuscik MJ, Rosier R, O'Keefe RJ, Schwarz EM, Awad HA (2011) Teriparatide therapy and beta-tricalcium phosphate enhance scaffold reconstruction of mouse femoral defects. *Tissue Eng Part A* **17**: 389-398.

Jensen TB, Overgaard S, Lind M, Rahbek O, Bunker C, Soballe K (2002) Osteogenic protein 1 device increases bone formation and bone graft resorption around cementless implants. *Acta Orthop Scand* **73**: 31-39.

Jeppsson C, Saveland H, Rydholm U, Aspenberg P (1999) OP-1 for cervical spine fusion: bridging bone in only 1 of 4 rheumatoid patients but prednisolone did not inhibit bone induction in rats. *Acta Orthop Scand* **70**: 559-563.

Jeyabalan J, Shah M, Viollet B, Roux JP, Chavassieux P, Korbonits M, Chenu C (2012) Mice lacking AMP-activated protein kinase $\alpha 1$ catalytic subunit have increased bone remodelling and modified skeletal responses to hormonal challenges induced by ovariectomy and intermittent PTH treatment. *J Endocrinol* **214**: 349-358.

Johnson EE, Urist MR, Finerman GA (1988a) Bone morphogenetic protein augmentation grafting of resistant femoral nonunions. A preliminary report. *Clin Orthop Relat Res* **230**: 257-265.

Johnson EE, Urist MR, Finerman GA (1988b) Repair of segmental defects of the tibia with cancellous bone grafts augmented with human bone morphogenetic protein. A preliminary report. *Clin Orthop Relat Res* **236**: 249-257.

Johnsson R, Stromqvist B, Aspenberg P (2002) Randomized radiostereometric study comparing osteogenic protein-1 (BMP-7) and autograft bone in human noninstrumented posterolateral lumbar fusion: 2002 Volvo Award in clinical studies. *Spine* **27**: 2654-2661.

Jones AL, Bucholz RW, Bosse MJ, Mirza SK, Lyon TR, Webb LX, Pollak AN, Golden JD, Valentin-Opran A (2006) Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction of diaphyseal tibial fractures with cortical defects. A randomized, controlled trial. *J Bone Joint Surg Am* **88**: 1431-1441.

Jung RE, Cochran DL, Domken O, Seibl R, Jones AA, Buser D, Hammerle CH (2007a) The effect of matrix bound parathyroid hormone on bone regeneration. *Clin Oral Implants Res* **18**: 319-325.

Jung RE, Hammerle CH, Kokovic V, Weber FE (2007b) Bone regeneration using a synthetic matrix containing a parathyroid hormone peptide combined with a grafting material. *Int J Oral Maxillofac Implants* **22**: 258-266.

Jung RE, Weber FE, Thoma DS, Ehrbar M, Cochran DL, Hammerle CH (2008) Bone morphogenetic protein-2 enhances bone formation when delivered by a synthetic matrix containing hydroxyapatite/tricalciumphosphate. *Clin Oral Implants Res* **19**: 188-195.

Kaback LA, Soung do Y, Naik A, Geneau G, Schwarz EM, Rosier RN, O'Keefe RJ, Drissi H (2008) Teriparatide (1-34 human PTH) regulation of osterix during fracture repair. *J Cell Biochem* **105**: 219-226.

Kaipel M, Schutzenberger S, Schultz A, Ferguson J, Slezak P, Morton TJ, Van Griensven M, Redl H (2012) BMP-2 but not VEGF or PDGF in fibrin matrix supports bone healing in a delayed-union rat model. *J Orthop Res* **30**: 1563-1569.

Kaito T, Myoui A, Takaoka K, Saito N, Nishikawa M, Tamai N, Ohgushi H, Yoshikawa H (2005) Potentiation of the activity of bone morphogenetic protein-2 in bone regeneration by a PLA-PEG/hydroxyapatite composite. *Biomaterials* **26**: 73-79.

Kakar S, Einhorn TA, Vora S, Miara LJ, Hon G, Wigner NA, Toben D, Jacobsen KA, Al-Sebaei MO, Song M, Trackman PC, Morgan EF, Gerstenfeld LC, Barnes GL (2007) Enhanced chondrogenesis and Wnt signaling in PTH-treated fractures. *J Bone Miner Res* **22**: 1903-1912.

Kallai I, van Lenthe GH, Ruffoni D, Zilberman Y, Muller R, Pelled G, Gazit D (2010) Quantitative, structural, and image-based mechanical analysis of nonunion fracture repaired by genetically engineered mesenchymal stem cells. *J Biomech* **43**: 2315-2320.

Kanakaris NK, Giannoudis PV (2007) The health economics of the treatment of long-bone non-unions. *Injury* **38 Suppl 2**: S77-84.

Kanakaris NK, Calori GM, Verdonk R, Burssens P, De Biase P, Capanna R, Vangosa LB, Cherubino P, Baldo F, Ristiniemi J, Kontakis G, Giannoudis PV (2008) Application of BMP-7 to tibial non-unions: a 3-year multicenter experience. *Injury* **39 Suppl 2**: S83-90.

Kanakaris NK, Lasanianos N, Calori GM, Verdonk R, Blokhuis TJ, Cherubino P, De Biase P, Giannoudis PV (2009) Application of bone morphogenetic proteins to femoral non-unions: a 4-year multicentre experience. *Injury* **40 Suppl 3**: S54-61.

Kanayama M, Hashimoto T, Shigenobu K, Yamane S, Bauer TW, Togawa D (2006) A prospective randomized study of posterolateral lumbar fusion using osteogenic protein-1 (OP-1) versus local autograft with ceramic bone substitute: emphasis of surgical exploration and histologic assessment. *Spine* **31**: 1067-1074.

Kanczler JM, Ginty PJ, Barry JJ, Clarke NM, Howdle SM, Shakesheff KM, Oreffo RO (2008) The effect of mesenchymal populations and vascular endothelial growth factor delivered from biodegradable polymer scaffolds on bone formation. *Biomaterials* **29**: 1892-1900.

Kanczler JM, Ginty PJ, White L, Clarke NM, Howdle SM, Shakesheff KM, Oreffo RO (2010) The effect of the delivery of vascular endothelial growth factor and bone morphogenetic protein-2 to osteoprogenitor cell populations on bone formation. *Biomaterials* **31**: 1242-1250.

Kanczler JM, Smith EL, Roberts CA, Oreffo RO (2012) A novel approach for studying the temporal modulation of embryonic skeletal development using organotypic bone cultures and microcomputed tomography. *Tissue Eng Part C Methods* **18**: 747-760.

Kanis JA, Johnell O (2005) Requirements for DXA for the management of osteoporosis in Europe. *Osteoporos Int* **16**: 229-238.

Karageorgiou V, Kaplan D (2005) Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials* **26**: 5474-5491.

- Karageorgiou V, Tomkins M, Fajardo R, Meinel L, Snyder B, Wade K, Chen J, Vunjak-Novakovic G, Kaplan DL (2006) Porous silk fibroin 3-D scaffolds for delivery of bone morphogenetic protein-2 *in vitro* and *in vivo*. *J Biomed Mater Res A* **78**: 324-334.
- Karaplis AC (2001) PTHrP: novel roles in skeletal biology. *Curr Pharm Des* **7**: 655-670.
- Katayama Y, Matsuyama Y, Yoshihara H, Sakai Y, Nakamura H, Imagama S, Ito Z, Wakao N, Kamiya M, Yukawa Y, Kanemura T, Sato K, Iwata H, Ishiguro N (2009) Clinical and radiographic outcomes of posterolateral lumbar spine fusion in humans using recombinant human bone morphogenetic protein-2: an average five-year follow-up study. *Int Orthop* **33**: 1061-1067.
- Kawai M, Bessho K, Maruyama H, Miyazaki J, Yamamoto T (2006) Simultaneous gene transfer of bone morphogenetic protein (BMP) -2 and BMP-7 by *in vivo* electroporation induces rapid bone formation and BMP-4 expression. *BMC Musculoskelet Disord* **7**: 62.
- Kawai M, Maruyama H, Bessho K, Yamamoto H, Miyazaki J, Yamamoto T (2009) Simple strategy for bone regeneration with a BMP-2/7 gene expression cassette vector. *Biochem Biophys Res Commun* **390**: 1012-1017.
- Keaveny TM, Hoffmann PF, Singh M, Palermo L, Bilezikian JP, Greenspan SL, Black DM (2008) Femoral bone strength and its relation to cortical and trabecular changes after treatment with PTH, alendronate, and their combination as assessed by finite element analysis of quantitative CT scans. *J Bone Miner Res* **23**: 1974-1982.
- Keaveny TM, McClung MR, Wan X, Kopperdahl DL, Mitlak BH, Krohn K (2012) Femoral strength in osteoporotic women treated with teriparatide or alendronate. *Bone* **50**: 165-170.
- Keene GS, Parker MJ, Pryor GA (1993) Mortality and morbidity after hip fractures. *BMJ* **307**: 1248-1250.
- Keeney M, van den Beucken JJ, van der Kraan PM, Jansen JA, Pandit A (2010) The ability of a collagen/calcium phosphate scaffold to act as its own vector for gene delivery and to promote bone formation *via* transfection with VEGF(165). *Biomaterials* **31**: 2893-2902.
- Kelpke SS, Zinn KR, Rue LW, Thompson JA (2004) Site-specific delivery of acidic fibroblast growth factor stimulates angiogenic and osteogenic responses *in vivo*. *J Biomed Mater Res A* **71**: 316-325.
- Kempen DH, Lu L, Heijink A, Hefferan TE, Creemers LB, Maran A, Yaszemski MJ, Dhert WJ (2009) Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration. *Biomaterials* **30**: 2816-2825.
- Kempen DH, Lu L, Hefferan TE, Creemers LB, Heijink A, Maran A, Dhert WJ, Yaszemski MJ (2010) Enhanced bone morphogenetic protein-2-induced ectopic and orthotopic bone formation by intermittent parathyroid hormone (1-34) administration. *Tissue Eng Part A* **16**: 3769-3777.
- Kenley R, Marden L, Turek T, Jin L, Ron E, Hollinger JO (1994) Osseous regeneration in the rat calvarium using novel delivery systems for recombinant human bone morphogenetic protein-2 (rhBMP-2). *J Biomed Mater Res* **28**: 1139-1147.
- Khan SN, Lane JM (2004) Spinal fusion surgery: animal models for tissue-engineered bone constructs. *Biomaterials* **25**: 1475-1485.
- Kidder LS, Chen X, Schmidt AH, Lew WD (2009) Osteogenic protein-1 overcomes inhibition of fracture healing in the diabetic rat: a pilot study. *Clin Orthop Relat Res* **467**: 3249-3256.
- Kim HW, Jahng JS (1999) Effect of intermittent administration of parathyroid hormone on fracture healing in ovariectomized rats. *Iowa Orthop J* **19**: 71-77.
- Kim CH, Takai E, Zhou H, von Stechow D, Muller R, Dempster DW, Guo XE (2003) Trabecular bone response to mechanical and parathyroid hormone stimulation: the role of mechanical microenvironment. *J Bone Miner Res* **18**: 2116-2125.
- Kim SS, Gwak SJ, Kim BS (2008) Orthotopic bone formation by implantation of apatite-coated poly(lactide-co-glycolide)/hydroxyapatite composite particulates and bone morphogenetic protein-2. *J Biomed Mater Res A* **87**: 245-253.
- Kim HK, Shim WS, Kim SE, Lee KH, Kang E, Kim JH, Kim K, Kwon IC, Lee DS (2009) Injectable *in situ*-forming pH/thermo-sensitive hydrogel for bone tissue engineering. *Tissue Eng Part A* **15**: 923-933.
- Kim HM, Galatz LM, Das R, Havlioglu N, Rothermich SY, Thomopoulos S (2010a) The role of transforming growth factor beta isoforms in tendon-to-bone healing. *Connect Tissue Res* **52**: 87-98.
- Kim PD, Ludwig S, Poelstra K, Duggan B, Scalea T, Gelb D (2010b) Ectopic bone formation in the pelvis after combined anterior and posterior fusion of the spine with osteogenic protein-1 use: a case report. *J Spinal Disord Tech* **23**: 215-220.
- Kim SJ, Shin HS, Shin SW (2010c) Effect of bone block graft with rhBMP-2 on vertical bone augmentation. *Int J Oral Maxillofac Surg* **39**: 883-888.
- Kim J, Sharma A, Runge B, Waters H, Doll B, McBride S, Alvarez P, Dadsetan M, Yaszemski MJ, Hollinger JO (2012) Osteoblast growth and bone-healing response to three-dimensional poly(epsilon-caprolactone fumarate) scaffolds. *J Tissue Eng Regen Med* **6**: 404-413.
- Kimelman-Bleich N, Pelled G, Sheyn D, Kallai I, Zilberman Y, Mizrahi O, Tal Y, Tawackoli W, Gazit Z, Gazit D (2009) The use of a synthetic oxygen carrier-enriched hydrogel to enhance mesenchymal stem cell-based bone formation *in vivo*. *Biomaterials* **30**: 4639-4648.
- Kimmel DB, Jee WS (1982) A quantitative histologic study of bone turnover in young adult beagles. *Anat Rec* **203**: 31-45.
- Kimura Y, Miyazaki N, Hayashi N, Otsuru S, Tamai K, Kaneda Y, Tabata Y (2010) Controlled release of bone morphogenetic protein-2 enhances recruitment of osteogenic progenitor cells for *de novo* generation of bone tissue. *Tissue Eng Part A* **16**: 1263-1270.
- Kinsella CR, Jr., Bykowski MR, Lin AY, Cray JJ, Durham EL, Smith DM, DeCesare GE, Mooney MP, Cooper GM, Losee JE (2011) BMP-2-mediated regeneration of large-scale cranial defects in the canine: an examination of different carriers. *Plast Reconstr Surg* **127**: 1865-1873.

- Kirker-Head CA, Gerhart TN, Armstrong R, Schelling SH, Carmel LA (1998) Healing bone using recombinant human bone morphogenetic protein 2 and copolymer. *Clin Orthop Relat Res* **349**: 205-217.
- Klimo P, Jr, Peelle MW (2009) Use of polyetheretherketone spacer and recombinant human bone morphogenetic protein-2 in the cervical spine: a radiographic analysis. *Spine J* **9**: 959-966.
- Kobayashi T, Chung UI, Schipani E, Starbuck M, Karsenty G, Katagiri T, Goad DL, Lanske B, Kronenberg HM (2002) PTHrP and Indian hedgehog control differentiation of growth plate chondrocytes at multiple steps. *Development* **129**: 2977-2986.
- Kodama N, Nagata M, Tabata Y, Ozeki M, Ninomiya T, Takagi R (2009) A local bone anabolic effect of rhFGF2-impregnated gelatin hydrogel by promoting cell proliferation and coordinating osteoblastic differentiation. *Bone* **44**: 699-707.
- Koh JT, Zhao Z, Wang Z, Lewis IS, Krebsbach PH, Franceschi RT (2008) Combinatorial gene therapy with BMP2/7 enhances cranial bone regeneration. *J Dent Res* **87**: 845-849.
- Kokubo S, Mochizuki M, Fukushima S, Ito T, Nozaki K, Iwai T, Takahashi K, Yokota S, Miyata K, Sasaki N (2004) Long-term stability of bone tissues induced by an osteoinductive biomaterial, recombinant human bone morphogenetic protein-2 and a biodegradable carrier. *Biomaterials* **25**: 1795-1803.
- Kolambkar YM, Boerckel JD, Dupont KM, Bajin M, Huebsch N, Mooney DJ, Hutmacher DW, Guldberg RE (2011) Spatiotemporal delivery of bone morphogenetic protein enhances functional repair of segmental bone defects. *Bone* **49**: 485-492.
- Komaki H, Tanaka T, Chazono M, Kikuchi T (2006) Repair of segmental bone defects in rabbit tibiae using a complex of beta-tricalcium phosphate, type I collagen, and fibroblast growth factor-2. *Biomaterials* **27**: 5118-5126.
- Komatsu DE, Brune KA, Liu H, Schmidt AL, Han B, Zeng QQ, Yang X, Nunes JS, Lu Y, Geiser AG, Ma YL, Wolos JA, Westmore MS, Sato M (2009) Longitudinal *in vivo* analysis of the region-specific efficacy of parathyroid hormone in a rat cortical defect model. *Endocrinology* **150**: 1570-1579.
- Komatsubara S, Mori S, Mashiba T, Nonaka K, Seki A, Akiyama T, Miyamoto K, Cao Y, Manabe T, Norimatsu H (2005) Human parathyroid hormone (1-34) accelerates the fracture healing process of woven to lamellar bone replacement and new cortical shell formation in rat femora. *Bone* **36**: 678-687.
- Komrakova M, Krischek C, Wicke M, Sehmisch S, Tezval M, Rohrberg M, Brandsch T, Stuermer KM, Stuermer EK (2011) Influence of intermittent administration of parathyroid hormone on muscle tissue and bone healing in orchietomized rats or controls. *J Endocrinol* **209**: 9-19.
- Kovacevic D, Fox AJ, Bedi A, Ying L, Deng XH, Warren RF, Rodeo SA (2011) Calcium-phosphate matrix with or without TGF-beta3 improves tendon-bone healing after rotator cuff repair. *Am J Sports Med* **39**: 811-819.
- Kraiwananapong C, Boden SD, Louis-Ugbo J, Attallah E, Barnes B, Hutton WC (2005) Comparison of Healos/bone marrow to INFUSE(rhBMP-2/ACS) with a collagen-ceramic sponge bulking agent as graft substitutes for lumbar spine fusion. *Spine* **30**: 1001-1007.
- Kuchler U, Luvizuto ER, Tangl S, Watzek G, Gruber R (2011) Short-term teriparatide delivery and osseointegration: a clinical feasibility study. *J Dent Res* **90**: 1001-1006.
- Kumar S, Nagy TR, Ponnazhagan S (2010a) Therapeutic potential of genetically modified adult stem cells for osteopenia. *Gene Ther* **17**: 105-116.
- Kumar S, Wan C, Ramaswamy G, Clemens TL, Ponnazhagan S (2010b) Mesenchymal stem cells expressing osteogenic and angiogenic factors synergistically enhance bone formation in a mouse model of segmental bone defect. *Mol Ther* **18**: 1026-1034.
- Kwan MD, Sellmyer MA, Quarto N, Ho AM, Wandless TJ, Longaker MT (2011) Chemical control of FGF-2 release for promoting calvarial healing with adipose stem cells. *J Biol Chem* **286**: 11307-11313.
- Kwon YD, Lee DW, Choi BJ, Lee JW, Kim DY (2012) Short-term teriparatide therapy as an adjunctive modality for bisphosphonate-related osteonecrosis of the jaws. *Osteoporos Int* **23**: 2721-2725.
- Lamerigts NM, Buma P, Huiskes R, Schreurs W, Gardeniers J, Slooff TJ (2000) Incorporation of morsellized bone graft under controlled loading conditions. A new animal model in the goat. *Biomaterials* **21**: 741-747.
- Lammens J, Nijs J, Schepers E, Ectors N, Lismont D, Verduyck B (2009) The effect of bone morphogenetic protein-7 (OP-1) and demineralized bone matrix (DBM) in the rabbit tibial distraction model. *Acta Orthop Belg* **75**: 103-109.
- Lane NE, Kumer J, Yao W, Breunig T, Wronski T, Modin G, Kinney JH (2003a) Basic fibroblast growth factor forms new trabeculae that physically connect with pre-existing trabeculae, and this new bone is maintained with an anti-resorptive agent and enhanced with an anabolic agent in an osteopenic rat model. *Osteoporos Int* **14**: 374-382.
- Lane NE, Yao W, Kinney JH, Modin G, Balooch M, Wronski TJ (2003b) Both hPTH(1-34) and bFGF increase trabecular bone mass in osteopenic rats but they have different effects on trabecular bone architecture. *J Bone Miner Res* **18**: 2105-2115.
- Laschke MW, Rucker M, Jensen G, Carvalho C, Mulhaupt R, Gellrich NC, Menger MD (2008) Incorporation of growth factor containing Matrigel promotes vascularization of porous PLGA scaffolds. *J Biomed Mater Res A* **85**: 397-407.
- Laursen M, Hoy K, Hansen ES, Gelineck J, Christensen FB, Bunger CE (1999) Recombinant bone morphogenetic protein-7 as an intracorporal bone growth stimulator in unstable thoracolumbar burst fractures in humans: preliminary results. *Eur Spine J* **8**: 485-490.
- Lawrence JP, Ennis F, White AP, Magit D, Polzhofer G, Drespe I, Troiano NW, Grauer JN (2006) Effect of daily parathyroid hormone (1-34) on lumbar fusion in a rat model. *Spine J* **6**: 385-390.
- Lazard ZW, Heggeness MH, Hipp JA, Sonnet C, Fuentes AS, Nistal RP, Davis AR, Olabisi RM, West JL, Olmsted-Davis EA (2011) Cell-based gene therapy

for repair of critical size defects in the rat fibula. *J Cell Biochem* **112**: 1563-1571.

Lee YM, Park YJ, Lee SJ, Ku Y, Han SB, Klokkevold PR, Chung CP (2000) The bone regenerative effect of platelet-derived growth factor-BB delivered with a chitosan/tricalcium phosphate sponge carrier. *J Periodontol* **71**: 418-424.

Lee JY, Musgrave D, Pelinkovic D, Fukushima K, Cummins J, Usas A, Robbins P, Fu FH, Huard J (2001a) Effect of bone morphogenetic protein-2-expressing muscle-derived cells on healing of critical-sized bone defects in mice. *J Bone Joint Surg Am* **83**: 1032-1039.

Lee SJ, Park YJ, Park SN, Lee YM, Seol YJ, Ku Y, Chung CP (2001b) Molded porous poly (L-lactide) membranes for guided bone regeneration with enhanced effects by controlled growth factor release. *J Biomed Mater Res* **55**: 295-303.

Lee JY, Nam SH, Im SY, Park YJ, Lee YM, Seol YJ, Chung CP, Lee SJ (2002) Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials. *J Control Release* **78**: 187-197.

Lee JW, Kim YH, Kim SH, Han SH, Hahn SB (2004) Chondrogenic differentiation of mesenchymal stem cells and its clinical applications. *Yonsei Med J* **45 Suppl**: 41-47.

Lee FY, Sinicropi SM, Lee FS, Vitale MG, Roye DP, Jr, Choi IH (2006) Treatment of congenital pseudarthrosis of the tibia with recombinant human bone morphogenetic protein-7 (rhBMP-7). A report of five cases. *J Bone Joint Surg Am* **88**: 627-633.

Lee SJ, Kang SW, Do HJ, Han I, Shin DA, Kim JH, Lee SH (2010) Enhancement of bone regeneration by gene delivery of BMP2/Runx2 bicistronic vector into adipose-derived stromal cells. *Biomaterials* **31**: 5652-5659.

Lee KB, Murray SS, Taghavi CE, Song KJ, Brochmann EJ, Johnson JS, Keorochana G, Liao JC, Wang JC (2011) Bone morphogenetic protein-binding peptide reduces the inflammatory response to recombinant human bone morphogenetic protein-2 and recombinant human bone morphogenetic protein-7 in a rodent model of soft-tissue inflammation. *Spine J* **11**: 568-576.

Lee YJ, Lee JH, Cho HJ, Kim HK, Yoon TR, Shin H (2013) Electrospun fibers immobilized with bone forming peptide-1 derived from BMP7 for guided bone regeneration. *Biomaterials* **34**: 5059-5069.

Lehman RA, Jr., Dmitriev AE, Cardoso MJ, Helgeson MD, Christensen CL, Raymond JW, Eckel TT, Riew KD (2010) Effect of teriparatide [rhPTH(1,34)] and calcitonin on intertransverse process fusion in a rabbit model. *Spine* **35**: 146-152.

Leknes KN, Yang J, Qahash M, Polimeni G, Susin C, Wikesjo UM (2008) Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-7/rhOP-1): radiographic observations. *J Clin Periodontol* **35**: 914-919.

Lendlein A, Langer R (2002) Biodegradable, elastic shape-memory polymers for potential biomedical applications. *Science* **296**: 1673-1676.

Leslie WD, Morin S, Lix LM, Johansson H, Oden A, McCloskey E, Kanis JA (2012) Fracture risk assessment without bone density measurement in routine clinical practice. *Osteoporos Int* **23**: 75-85.

Leung KS, Siu WS, Cheung NM, Lui PY, Chow DH, James A, Qin L (2001) Goats as an osteopenic animal model. *J Bone Miner Res* **16**: 2348-2355.

Levi B, James AW, Nelson ER, Vistnes D, Wu B, Lee M, Gupta A, Longaker MT (2010) Human adipose derived stromal cells heal critical size mouse calvarial defects. *PLoS One* **5**: e11177.

Li M, Healy DR, Li Y, Simmons HA, Gao F, Ke HZ, Lu B, Owen TA, Thompson DD (2001) A comparison of the anabolic effects of rat and bovine parathyroid hormone (1-34) in ovariectomized rats. *J Musculoskelet Neuronal Interact* **2**: 77-83.

Li RH, Bouxsein ML, Blake CA, D'Augusta D, Kim H, Li XJ, Wozney JM, Seeherman HJ (2003) rhBMP-2 injected in a calcium phosphate paste (alpha-BSM) accelerates healing in the rabbit ulnar osteotomy model. *J Orthop Res* **21**: 997-1004.

Li G, Corsi-Payne K, Zheng B, Usas A, Peng H, Huard J (2009a) The dose of growth factors influences the synergistic effect of vascular endothelial growth factor on bone morphogenetic protein 4-induced ectopic bone formation. *Tissue Eng Part A* **15**: 2123-2133.

Li R, Stewart DJ, von Schroeder HP, Mackinnon ES, Schemitsch EH (2009b) Effect of cell-based VEGF gene therapy on healing of a segmental bone defect. *J Orthop Res* **27**: 8-14.

Li J, Li Y, Ma S, Gao Y, Zuo Y, Hu J (2010a) Enhancement of bone formation by BMP-7 transduced MSCs on biomimetic nano-hydroxyapatite/polyamide composite scaffolds in repair of mandibular defects. *J Biomed Mater Res A* **95**: 973-981.

Li M, Liu X, Ge B (2010b) Calcium phosphate cement with BMP-2-loaded gelatin microspheres enhances bone healing in osteoporosis: a pilot study. *Clin Orthop Relat Res* **468**: 1978-1985.

Li YF, Li XD, Bao CY, Chen QM, Zhang H, Hu J (2013) Promotion of peri-implant bone healing by systemically administered parathyroid hormone (1-34) and zoledronic acid adsorbed onto the implant surface. *Osteoporos Int* **24**: 1063-1071.

Lieberman JR, Daluiski A, Stevenson S, Wu L, McAllister P, Lee YP, Kabo JM, Finerman GA, Berk AJ, Witte ON (1999) The effect of regional gene therapy with bone morphogenetic protein-2-producing bone-marrow cells on the repair of segmental femoral defects in rats. *J Bone Joint Surg Am* **81**: 905-917.

Liebschner MA (2004) Biomechanical considerations of animal models used in tissue engineering of bone. *Biomaterials* **25**: 1697-1714.

Lin Y, Tang W, Wu L, Jing W, Li X, Wu Y, Liu L, Long J, Tian W (2008) Bone regeneration by BMP-2 enhanced adipose stem cells loading on alginate gel. *Histochem Cell Biol* **129**: 203-210.

Lin CY, Chang YH, Lin KJ, Yen TC, Tai CL, Chen CY, Lo WH, Hsiao IT, Hu YC (2010) The healing of critical-sized femoral segmental bone defects in rabbits using baculovirus-engineered mesenchymal stem cells. *Biomaterials* **31**: 3222-3230.

Lin CY, Chang YH, Kao CY, Lu CH, Sung LY, Yen TC, Lin KJ, Hu YC (2012a) Augmented healing of critical-size calvarial defects by baculovirus-engineered MSCs

that persistently express growth factors. *Biomaterials* **33**: 3682-3692.

Lin EA, Liu CJ, Monroy A, Khurana S, Egol KA (2012b) Prevention of atrophic nonunion by the systemic administration of parathyroid hormone (PTH 1-34) in an experimental animal model. *J Orthop Trauma* **26**: 719-723.

Lin Z, Rios HF, Park CH, Taut AD, Jin Q, Sugai JV, Robbins PD, Giannobile WV (2012c) LIM domain protein-3 (LMP3) cooperates with BMP7 to promote tissue regeneration by ligament progenitor cells. *Gene Ther* **20**: 1-6.

Lind M, Overgaard S, Jensen TB, Song Y, Goodman SB, Bunger C, Soballe K (2001) Effect of osteogenic protein 1/collagen composite combined with impacted allograft around hydroxyapatite-coated titanium alloy implants is moderate. *J Biomed Mater Res* **55**: 89-95.

Lindsey RW, Gugala Z, Milne E, Sun M, Gannon FH, Latta LL (2006) The efficacy of cylindrical titanium mesh cage for the reconstruction of a critical-size canine segmental femoral diaphyseal defect. *J Orthop Res* **24**: 1438-1453.

Liu G, Vijayakumar S, Grumolato L, Arroyave R, Qiao H, Akiri G, Aaronson SA (2009) Canonical Wnts function as potent regulators of osteogenesis by human mesenchymal stem cells. *J Cell Biol* **185**: 67-75.

Liu F, Porter RM, Wells J, Glatt V, Pilapil C, Evans CH (2012) Evaluation of BMP-2 gene-activated muscle grafts for cranial defect repair. *J Orthop Res* **30**: 1095-1102.

Liu SS, Xu H, Sun J, Kontogiorgos E, Whittington PR, Misner KG, Kyung HM, Buschang PH, Opperman LA (2013) Recombinant human bone morphogenetic protein-2 stimulates bone formation during interfrontal suture expansion in rabbits. *Am J Orthod Dentofacial Orthop* **144**: 210-217.

Lozano D, de Castro LF, Dapia S, Andrade-Zapata I, Manzarbeitia F, Alvarez-Arroyo MV, Gomez-Barrena E, Esbrit P (2009) Role of parathyroid hormone-related protein in the decreased osteoblast function in diabetes-related osteopenia. *Endocrinology* **150**: 2027-2035.

Lozano D, Fernandez-de-Castro L, Portal-Nunez S, Lopez-Herradon A, Dapia S, Gomez-Barrena E, Esbrit P (2010) The C-terminal fragment of parathyroid hormone-related peptide promotes bone formation in diabetic mice with low-turnover osteopaenia. *Br J Pharmacol* **162**: 1424-1438.

Luca L, Rougemont AL, Walpoth BH, Boure L, Tami A, Anderson JM, Jordan O, Gurny R (2010a) Injectable rhBMP-2-loaded chitosan hydrogel composite: osteoinduction at ectopic site and in segmental long bone defect. *J Biomed Mater Res A* **96**: 66-74.

Luca L, Rougemont AL, Walpoth BH, Gurny R, Jordan O (2010b) The effects of carrier nature and pH on rhBMP-2-induced ectopic bone formation. *J Control Release* **147**: 38-44.

Ma PX (2008) Biomimetic materials for tissue engineering. *Adv Drug Deliv Rev* **60**: 184-198.

Ma YL, Cain RL, Halladay DL, Yang X, Zeng Q, Miles RR, Chandrasekhar S, Martin TJ, Onyia JE (2001) Catabolic effects of continuous human PTH (1--38) *in vivo* is associated with sustained stimulation of RANKL and

inhibition of osteoprotegerin and gene-associated bone formation. *Endocrinology* **142**: 4047-4054.

Ma T, Gutnick J, Salazar B, Larsen MD, Suenaga E, Zilber S, Huang Z, Huddleston J, Smith RL, Goodman S (2007) Modulation of allograft incorporation by continuous infusion of growth factors over a prolonged duration *in vivo*. *Bone* **41**: 386-392.

Madry H, Orth P, Kaul G, Zurakowski D, Menger MD, Kohn D, Cucchiari M (2010) Acceleration of articular cartilage repair by combined gene transfer of human insulin-like growth factor I and fibroblast growth factor-2 *in vivo*. *Arch Orthop Trauma Surg* **130**: 1311-1322.

Maehara H, Sotome S, Yoshii T, Torigoe I, Kawasaki Y, Sugata Y, Yuasa M, Hirano M, Mochizuki N, Kikuchi M, Shinomiya K, Okawa A (2009) Repair of large osteochondral defects in rabbits using porous hydroxyapatite/collagen (HAp/Col) and fibroblast growth factor-2 (FGF-2). *J Orthop Res* **28**: 677-686.

Magin MN, Delling G (2001) Improved lumbar vertebral interbody fusion using rhOP-1: a comparison of autogenous bone graft, bovine hydroxylapatite (Bio-Oss), and BMP-7 (rhOP-1) in sheep. *Spine* **26**: 469-478.

Makino T, Hak DJ, Hazelwood SJ, Curtiss S, Reddi AH (2005) Prevention of atrophic nonunion development by recombinant human bone morphogenetic protein-7. *J Orthop Res* **23**: 632-638.

Manabe T, Mori S, Mashiba T, Kaji Y, Iwata K, Komatsubara S, Seki A, Sun YX, Yamamoto T (2007) Human parathyroid hormone (1-34) accelerates natural fracture healing process in the femoral osteotomy model of cynomolgus monkeys. *Bone* **40**: 1475-1482.

Marden LJ, Fan RS, Pierce GF, Reddi AH, Hollinger JO (1993) Platelet-derived growth factor inhibits bone regeneration induced by osteogenin, a bone morphogenetic protein, in rat craniotomy defects. *J Clin Invest* **92**: 2897-2905.

Marden LJ, Hollinger JO, Chaudhari A, Turek T, Schaub RG, Ron E (1994) Recombinant human bone morphogenetic protein-2 is superior to demineralized bone matrix in repairing craniotomy defects in rats. *J Biomed Mater Res* **28**: 1127-1138.

Marques MR, da Silva MA, Manzi FR, Cesar-Neto JB, Nociti FH, Jr, Barros SP (2005) Effect of intermittent PTH administration in the periodontitis-associated bone loss in ovariectomized rats. *Arch Oral Biol* **50**: 421-429.

Martino MM, Tortelli F, Mochizuki M, Traub S, Ben-David D, Kuhn GA, Muller R, Livne E, Eming SA, Hubbell JA (2011) Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. *Sci Transl Med* **3**: 100ra189.

Marukawa E, Asahina I, Oda M, Seto I, Alam MI, Enomoto S (2001) Bone regeneration using recombinant human bone morphogenetic protein-2 (rhBMP-2) in alveolar defects of primate mandibles. *Br J Oral Maxillofac Surg* **39**: 452-459.

Marukawa E, Asahina I, Oda M, Seto I, Alam M, Enomoto S (2002) Functional reconstruction of the non-human primate mandible using recombinant human bone morphogenetic protein-2. *Int J Oral Maxillofac Surg* **31**: 287-295.

- Maus U, Andereya S, Gravius S, Ohnsorge JA, Niedhart C, Siebert CH (2008) BMP-2 incorporated in a tricalcium phosphate bone substitute enhances bone remodeling in sheep. *J Biomater Appl* **22**: 559-576.
- Mayahara H, Ito T, Nagai H, Miyajima H, Tsukuda R, Taketomi S, Mizoguchi J, Kato K (1993) *In vivo* stimulation of endosteal bone formation by basic fibroblast growth factor in rats. *Growth Factors* **9**: 73-80.
- Mayer M, Hollinger J, Ron E, Wozney J (1996) Maxillary alveolar cleft repair in dogs using recombinant human bone morphogenetic protein-2 and a polymer carrier. *Plast Reconstr Surg* **98**: 247-259.
- McGee MA, Findlay DM, Howie DW, Carbone A, Ward P, Stamenkov R, Page TT, Bruce WJ, Wildenauer CI, Toth C (2004) The use of OP-1 in femoral impaction grafting in a sheep model. *J Orthop Res* **22**: 1008-1015.
- McKay WF, Peckham SM, Badura JM (2007) A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). *Int Orthop* **31**: 729-734.
- McMahon LA, O'Brien FJ, Prendergast PJ (2008) Biomechanics and mechanobiology in osteochondral tissues. *Regen Med* **3**: 743-759.
- Menendez MI, Clark DJ, Carlton M, Flanagan DC, Jia G, Sammet S, Weisbrode SE, Knopp MV, Bertone AL (2011) Direct delayed human adenoviral BMP-2 or BMP-6 gene therapy for bone and cartilage regeneration in a pony osteochondral model. *Osteoarthritis Cartilage* **19**: 1066-1075.
- Meng X, Baylink DJ, Sheng M, Wang H, Gridley DS, Lau KH, Zhang XB (2012) Erythroid promoter confines FGF2 expression to the marrow after hematopoietic stem cell gene therapy and leads to enhanced endosteal bone formation. *PLoS One* **7**: e37569.
- Meraw SJ, Reeve CM, Lohse CM, Sioussat TM (2000) Treatment of peri-implant defects with combination growth factor cement. *J Periodontol* **71**: 8-13.
- Mesfin A, Buchowski JM, Zebala LP, Bakhsh WR, Aronson AB, Fogelson JL, Hershtman S, Kim HJ, Ahmad A, Bridwell KH (2013) High-dose rhBMP-2 for adults: major and minor complications: a study of 502 spine cases. *J Bone Joint Surg Am* **95**: 1546-1553.
- Mimura T, Imai S, Okumura N, Li L, Nishizawa K, Araki S, Ueba H, Kubo M, Mori K, Matsusue Y (2011) Spatiotemporal control of proliferation and differentiation of bone marrow-derived mesenchymal stem cells recruited using collagen hydrogel for repair of articular cartilage defects. *J Biomed Mater Res B Appl Biomater* **98**: 360-368.
- Miner S, Leucht P, Jiang J, Liu B, Zeng A, Fuerer C, Nusse R, Helms JA (2010) Wnt proteins promote bone regeneration. *Sci Transl Med* **2**: 29ra30.
- Miyazaki M, Sugiyama O, Tow B, Zou J, Morishita Y, Wei F, Napoli A, Sintuu C, Lieberman JR, Wang JC (2008) The effects of lentiviral gene therapy with bone morphogenetic protein-2-producing bone marrow cells on spinal fusion in rats. *J Spinal Disord Tech* **21**: 372-379.
- Mizumoto Y, Moseley T, Drews M, Cooper VN, 3rd, Reddi AH (2003) Acceleration of regenerate ossification during distraction osteogenesis with recombinant human bone morphogenetic protein-7. *J Bone Joint Surg Am* **85 Suppl 3**: 124-130.
- Mladenov KV, Kunkel P, Stuecker R (2010) The use of recombinant human BMP-2 as a salvage procedure in the pediatric spine: a report on 3 cases. *Eur Spine J* **19 Suppl 2**: S135-139.
- Mohan S, Kutilek S, Zhang C, Shen HG, Kodama Y, Srivastava AK, Wergedal JE, Beamer WG, Baylink DJ (2000) Comparison of bone formation responses to parathyroid hormone(1-34), (1-31), and (2-34) in mice. *Bone* **27**: 471-478.
- Mont MA, Jones LC, Elias JJ, Inoue N, Yoon TR, Chao EY, Hungerford DS (2001) Strut-autografting with and without osteogenic protein-1 : a preliminary study of a canine femoral head defect model. *J Bone Joint Surg Am* **83**: 1013-1022.
- Moore DC, Ehrlich MG, McAllister SC, Machan JT, Hart CE, Voigt C, Lesieur-Brooks AM, Weber EW (2009) Recombinant human platelet-derived growth factor-BB augmentation of new-bone formation in a rat model of distraction osteogenesis. *J Bone Joint Surg Am* **91**: 1973-1984.
- Morgan EF, Mason ZD, Bishop G, Davis AD, Wigner NA, Gerstenfeld LC, Einhorn TA (2008) Combined effects of recombinant human BMP-7 (rhBMP-7) and parathyroid hormone (1-34) in metaphyseal bone healing. *Bone* **43**: 1031-1038.
- Mori M, Isobe M, Yamazaki Y, Ishihara K, Nakabayashi N (2000) Restoration of segmental bone defects in rabbit radius by biodegradable capsules containing recombinant human bone morphogenetic protein-2. *J Biomed Mater Res* **50**: 191-198.
- Mosekilde L, Weisbrode SE, Safron JA, Stills HF, Jankowsky ML, Ebert DC, Danielsen CC, Sogaard CH, Franks AF, Stevens ML (1993) Calcium-restricted ovariectomized Sinclair S-1 minipigs: an animal model of osteopenia and trabecular plate perforation. *Bone* **14**: 379-382.
- Moshel YA, Hernandez EI, Kong L, Liu C, Samadani U (2008) Acute renal insufficiency, supraventricular tachycardia, and confusion after recombinant human bone morphogenetic protein-2 implantation for lumbosacral spine fusion. *J Neurosurg Spine* **8**: 589-593.
- Mrugala D, Bony C, Neves N, Caillot L, Fabre S, Moukoko D, Jorgensen C, Noel D (2008) Phenotypic and functional characterisation of ovine mesenchymal stem cells: application to a cartilage defect model. *Ann Rheum Dis* **67**: 288-295.
- Mumaw J, Jordan ET, Sonnet C, Olabisi RM, Olmsted-Davis EA, Davis AR, Peroni JF, West JL, West F, Lu Y, Stice SL (2012) Rapid heterotrophic ossification with cryopreserved poly(ethylene glycol-) microencapsulated BMP2-expressing MSCs. *Int J Biomater* **2012**: 861794.
- Murakami N, Saito N, Horiuchi H, Okada T, Nozaki K, Takaoka K (2002) Repair of segmental defects in rabbit humeri with titanium fiber mesh cylinders containing recombinant human bone morphogenetic protein-2 (rhBMP-2) and a synthetic polymer. *J Biomed Mater Res* **62**: 169-174.
- Murakami N, Saito N, Takahashi J, Ota H, Horiuchi H, Nawata M, Okada T, Nozaki K, Takaoka K (2003) Repair of a proximal femoral bone defect in dogs using a porous surfaced prosthesis in combination with recombinant

BMP-2 and a synthetic polymer carrier. *Biomaterials* **24**: 2153-2159.

Murphy WL, Mooney DJ (1999) Controlled delivery of inductive proteins, plasmid DNA and cells from tissue engineering matrices. *J Periodontol Res* **34**: 413-419.

Musgrave DS, Bosch P, Ghivizzani S, Robbins PD, Evans CH, Huard J (1999) Adenovirus-mediated direct gene therapy with bone morphogenetic protein-2 produces bone. *Bone* **24**: 541-547.

Na K, Park JH, Kim SW, Sun BK, Woo DG, Chung HM, Park KH (2006) Delivery of dexamethasone, ascorbate, and growth factor (TGF beta-3) in thermo-reversible hydrogel constructs embedded with rabbit chondrocytes. *Biomaterials* **27**: 5951-5957.

Nagai H, Tsukuda R, Mayahara H (1995) Effects of basic fibroblast growth factor (bFGF) on bone formation in growing rats. *Bone* **16**: 367-373.

Nakajima A, Shimoji N, Shiomi K, Shimizu S, Moriya H, Einhorn TA, Yamazaki M (2002) Mechanisms for the enhancement of fracture healing in rats treated with intermittent low-dose human parathyroid hormone (1-34). *J Bone Miner Res* **17**: 2038-2047.

Nakamura T, Hara Y, Tagawa M, Tamura M, Yuge T, Fukuda H, Nigi H (1998) Recombinant human basic fibroblast growth factor accelerates fracture healing by enhancing callus remodeling in experimental dog tibial fracture. *J Bone Miner Res* **13**: 942-949.

Nakamura Y, Tensho K, Nakaya H, Nawata M, Okabe T, Wakitani S (2005) Low dose fibroblast growth factor-2 (FGF-2) enhances bone morphogenetic protein-2 (BMP-2)-induced ectopic bone formation in mice. *Bone* **36**: 399-407.

Nakamura T, Sugimoto T, Nakano T, Kishimoto H, Ito M, Fukunaga M, Hagino H, Sone T, Yoshikawa H, Nishizawa Y, Fujita T, Shiraki M (2012) Randomized Teriparatide [human parathyroid hormone (PTH) 1-34] Once-Weekly Efficacy Research (TOWER) trial for examining the reduction in new vertebral fractures in subjects with primary osteoporosis and high fracture risk. *J Clin Endocrinol Metab* **97**: 3097-3106.

Nakasa T, Ishida O, Sunagawa T, Nakamae A, Yasunaga Y, Agung M, Ochi M (2005) Prefabrication of vascularized bone graft using a combination of fibroblast growth factor-2 and vascular bundle implantation into a novel interconnected porous calcium hydroxyapatite ceramic. *J Biomed Mater Res A* **75**: 350-355.

Nakasa T, Ishida O, Sunagawa T, Nakamae A, Yokota K, Adachi N, Ochi M (2008) Feasibility of prefabricated vascularized bone graft using the combination of FGF-2 and vascular bundle implantation within hydroxyapatite for osteointegration. *J Biomed Mater Res A* **85**: 1090-1095.

Nalesso G, Sherwood J, Bertrand J, Pap T, Ramachandran M, De Bari C, Pitzalis C, Dell'Accio F (2011) WNT-3A modulates articular chondrocyte phenotype by activating both canonical and noncanonical pathways. *J Cell Biol* **193**: 551-564.

Nash TJ, Howlett CR, Martin C, Steele J, Johnson KA, Hicklin DJ (1994) Effect of platelet-derived growth factor on tibial osteotomies in rabbits. *Bone* **15**: 203-208.

Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH (2001) Effect of

parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* **344**: 1434-1441.

Nevins M, Camelo M, Nevins ML, Schenk RK, Lynch SE (2003) Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. *J Periodontol* **74**: 1282-1292.

Newman E, Turner AS, Wark JD (1995) The potential of sheep for the study of osteopenia: current status and comparison with other animal models. *Bone* **16**: 277S-284S.

Neyt JG, Buckwalter JA, Carroll NC (1998) Use of animal models in musculoskeletal research. *Iowa Orthop J* **18**: 118-123.

Nilsson OS, Urist MR, Dawson EG, Schmalzried TP, Finerman GA (1986) Bone repair induced by bone morphogenetic protein in ulnar defects in dogs. *J Bone Joint Surg Br* **68**: 635-642.

Niziolek PJ, Murthy S, Ellis SN, Sukhija KB, Hornberger TA, Turner CH, Robling AG (2009) Rapamycin impairs trabecular bone acquisition from high-dose but not low-dose intermittent parathyroid hormone treatment. *J Cell Physiol* **221**: 579-585.

Nociti Junior FH, Stefani CM, Machado MA, Sallum EA, Toledo S, Sallum AW (2000) Histometric evaluation of bone regeneration around immediate implants partially in contact with bone: a pilot study in dogs. *Implant Dent* **9**: 321-328.

Nozaka K, Miyakoshi N, Kasukawa Y, Maekawa S, Noguchi H, Shimada Y (2008) Intermittent administration of human parathyroid hormone enhances bone formation and union at the site of cancellous bone osteotomy in normal and ovariectomized rats. *Bone* **42**: 90-97.

Nussenbaum B, Rutherford RB, Teknos TN, Dornfeld KJ, Krebsbach PH (2003) *Ex vivo* gene therapy for skeletal regeneration in cranial defects compromised by postoperative radiotherapy. *Hum Gene Ther* **14**: 1107-1115.

O'Loughlin PF, Morr S, Bogunovic L, Kim AD, Park B, Lane JM (2008) Selection and development of preclinical models in fracture-healing research. *J Bone Joint Surg Am* **90 Suppl 1**: 79-84.

O'Loughlin PF, Cunningham ME, Bukata SV, Tomin E, Poynton AR, Doty SB, Sama AA, Lane JM (2009) Parathyroid hormone (1-34) augments spinal fusion, fusion mass volume, and fusion mass quality in a rabbit spinal fusion model. *Spine* **34**: 121-130.

O'Shaughnessy BA, Kuklo TR, Ondra SL (2008) Surgical treatment of vertebral osteomyelitis with recombinant human bone morphogenetic protein-2. *Spine* **33**: E132-139.

Oest ME, Dupont KM, Kong HJ, Mooney DJ, Guldberg RE (2007) Quantitative assessment of scaffold and growth factor-mediated repair of critically sized bone defects. *J Orthop Res* **25**: 941-950.

Ohura K, Hamanishi C, Tanaka S, Matsuda N (1998) Healing of segmental bone defects in rats induced by a beta-TCP-MCPM cement combined with rhBMP-2. *J Biomed Mater Res* **44**: 168-175.

Olabisi RM, Lazard ZW, Franco CL, Hall MA, Kwon SK, Seivick-Muraca EM, Hipp JA, Davis AR,

Olmsted-Davis EA, West JL (2010) Hydrogel microsphere encapsulation of a cell-based gene therapy system increases cell survival of injected cells, transgene expression, and bone volume in a model of heterotopic ossification. *Tissue Eng Part A* **16**: 3727-3736.

Ong KL, Villarraga ML, Lau E, Carreon LY, Kurtz SM, Glassman SD (2010) Off-label use of bone morphogenetic proteins in the United States using administrative data. *Spine* **35**: 1794-1800.

Onyia JE, Bidwell J, Herring J, Hulman J, Hock JM (1995) *In vivo*, human parathyroid hormone fragment (hPTH 1-34) transiently stimulates immediate early response gene expression, but not proliferation, in trabecular bone cells of young rats. *Bone* **17**: 479-484.

Opperman LA, Moursi AM, Sayne JR, Wintergerst AM (2002) Transforming growth factor-beta 3 (Tgf-beta3) in a collagen gel delays fusion of the rat posterior interfrontal suture *in vivo*. *Anat Rec* **267**: 120-130.

Ornitz DM, Itoh N (2001) Fibroblast growth factors. *Genome Biol* **2**: S3005.

Ornitz DM, Marie PJ (2002) FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. *Genes Dev* **16**: 1446-1465.

Osawa K, Okubo Y, Nakao K, Koyama N, Bessho K (2009) Osteoinduction by microbubble-enhanced transcutaneous sonoporation of human bone morphogenetic protein-2. *J Gene Med* **11**: 633-641.

Papanna MC, Al-Hadithy N, Somanchi BV, Sewell MD, Robinson PM, Khan SA, Wilkes RA (2012) The use of bone morphogenetic protein-7 (OP-1) in the management of resistant non-unions in the upper and lower limb. *Injury* **43**: 1135-1140.

Park YJ, Ku Y, Chung CP, Lee SJ (1998) Controlled release of platelet-derived growth factor from porous poly(L-lactide) membranes for guided tissue regeneration. *J Control Release* **51**: 201-211.

Park YJ, Lee YM, Park SN, Sheen SY, Chung CP, Lee SJ (2000) Platelet derived growth factor releasing chitosan sponge for periodontal bone regeneration. *Biomaterials* **21**: 153-159.

Park J, Ries J, Gelse K, Kloss F, von der Mark K, Wiltfang J, Neukam FW, Schneider H (2003) Bone regeneration in critical size defects by cell-mediated BMP-2 gene transfer: a comparison of adenoviral vectors and liposomes. *Gene Ther* **10**: 1089-1098.

Park J, Lutz R, Felszeghy E, Wiltfang J, Nkenke E, Neukam FW, Schlegel KA (2007) The effect on bone regeneration of a liposomal vector to deliver BMP-2 gene to bone grafts in peri-implant bone defects. *Biomaterials* **28**: 2772-2782.

Park JS, Woo DG, Yang HN, Na K, Park KH (2009) Transforming growth factor beta-3 bound with sulfate polysaccharide in synthetic extracellular matrix enhanced the biological activities for neocartilage formation *in vivo*. *J Biomed Mater Res A* **91**: 408-415.

Park JS, Woo DG, Yang HN, Lim HJ, Park KM, Na K, Park KH (2010a) Chondrogenesis of human mesenchymal stem cells encapsulated in a hydrogel construct: neocartilage formation in animal models as both mice and rabbits. *J Biomed Mater Res A* **92**: 988-996.

Park JS, Yang HJ, Woo DG, Yang HN, Na K, Park KH (2010b) Chondrogenic differentiation of mesenchymal stem cells embedded in a scaffold by long-term release of TGF-beta 3 complexed with chondroitin sulfate. *J Biomed Mater Res A* **92**: 806-816.

Park JS, Yang HN, Woo DG, Jeon SY, Park KH (2011) Chondrogenesis of human mesenchymal stem cells in fibrin constructs evaluated *in vitro* and in nude mouse and rabbit defects models. *Biomaterials* **32**: 1495-1507.

Park JS, Yang HN, Woo DG, Jeon SY, Park KH (2012) SOX9 gene plus heparinized TGF-beta 3 coated dexamethasone loaded PLGA microspheres for inducement of chondrogenesis of hMSCs. *Biomaterials* **33**: 7151-7163.

Park SY, Kim KH, Shin SY, Koo KT, Lee YM, Seol YJ (2013) Dual delivery of rhPDGF-BB and bone marrow mesenchymal stromal cells expressing the BMP2 gene enhance bone formation in a critical-sized defect model. *Tissue Eng Part A* **19**: 2495-2505.

Patel ZS, Young S, Tabata Y, Jansen JA, Wong ME, Mikos AG (2008) Dual delivery of an angiogenic and an osteogenic growth factor for bone regeneration in a critical size defect model. *Bone* **43**: 931-940.

Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG (2007) Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater* **13**: 1-10.

Peichl P, Holzer LA, Maier R, Holzer G (2011) Parathyroid hormone 1-84 accelerates fracture-healing in pubic bones of elderly osteoporotic women. *J Bone Joint Surg Am* **93**: 1583-1587.

Pettway GJ, Schneider A, Koh AJ, Widjaja E, Morris MD, Meganck JA, Goldstein SA, McCauley LK (2005) Anabolic actions of PTH (1-34): use of a novel tissue engineering model to investigate temporal effects on bone. *Bone* **36**: 959-970.

Pettway GJ, Meganck JA, Koh AJ, Keller ET, Goldstein SA, McCauley LK (2008) Parathyroid hormone mediates bone growth through the regulation of osteoblast proliferation and differentiation. *Bone* **42**: 806-818.

Phillips FM, Turner AS, Seim HB, 3rd, MacLeay J, Toth CA, Pierce AR, Wheeler DL (2006) *In vivo* BMP-7 (OP-1) enhancement of osteoporotic vertebral bodies in an ovine model. *Spine* **31**: 500-506.

Plotkin H, Gundberg C, Mitnick M, Stewart AF (1998) Dissociation of bone formation from resorption during 2-week treatment with human parathyroid hormone-related peptide-(1-36) in humans: potential as an anabolic therapy for osteoporosis. *J Clin Endocrinol Metab* **83**: 2786-2791.

Podbesek R, Edouard C, Meunier PJ, Parsons JA, Reeve J, Stevenson RW, Zanelli JM (1983) Effects of two treatment regimes with synthetic human parathyroid hormone fragment on bone formation and the tissue balance of trabecular bone in greyhounds. *Endocrinology* **112**: 1000-1006.

Portal-Nunez S, Lozano D, de Castro LF, de Gortazar AR, Nogues X, Esbrit P (2010) Alterations of the Wnt/beta-catenin pathway and its target genes for the N- and C-terminal domains of parathyroid hormone-related protein in bone from diabetic mice. *FEBS Lett* **584**: 3095-3100.

- Qiang YW, Shaughnessy JD, Jr., Yaccoby S (2008) Wnt3a signaling within bone inhibits multiple myeloma bone disease and tumor growth. *Blood* **112**: 374-382.
- Qing W, Guang-Xing C, Lin G, Liu Y (2012) The osteogenic study of tissue engineering bone with BMP2 and BMP7 gene-modified rat adipose-derived stem cell. *J Biomed Biotechnol* **2012**: 410879.
- Qiu Z, Wei L, Liu J, Sochacki KR, Liu X, Bishop C, Ebraheim M, Yang H (2013) Effect of intermittent PTH (1-34) on posterolateral spinal fusion with iliac crest bone graft in an ovariectomized rat model. *Osteoporos Int* **24**: 2693-2700.
- Qu D, Li J, Li Y, Gao Y, Zuo Y, Hsu Y, Hu J (2011) Angiogenesis and osteogenesis enhanced by bFGF *ex vivo* gene therapy for bone tissue engineering in reconstruction of calvarial defects. *J Biomed Mater Res A* **96**: 543-551.
- Quaglia F (2008) Bioinspired tissue engineering: the great promise of protein delivery technologies. *Int J Pharm* **364**: 281-297.
- Quesada-Gomez JM, Muschitz C, Gomez-Reino J, Greisen H, Andersen HS, Dimai HP (2011) The effect of PTH(1-84) or strontium ranelate on bone formation markers in postmenopausal women with primary osteoporosis: results of a randomized, open-label clinical trial. *Osteoporos Int* **22**: 2529-2537.
- Ramazanoglu M, Lutz R, Ergun C, von Wilmowsky C, Nkenke E, Schlegel KA (2011) The effect of combined delivery of recombinant human bone morphogenetic protein-2 and recombinant human vascular endothelial growth factor 165 from biomimetic calcium-phosphate-coated implants on osseointegration. *Clin Oral Implants Res* **22**: 1433-1439.
- Ranly DM, McMillan J, Keller T, Lohmann CH, Meunch T, Cochran DL, Schwartz Z, Boyan BD (2005) Platelet-derived growth factor inhibits demineralized bone matrix-induced intramuscular cartilage and bone formation. A study of immunocompromised mice. *J Bone Joint Surg Am* **87**: 2052-2064.
- Ravaglioli A, Krajewski A, Celotti GC, Piancastelli A, Bacchini B, Montanari L, Zama G, Piombi L (1996) Mineral evolution of bone. *Biomaterials* **17**: 617-622.
- Reddi AH (2000) Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. *Tissue Eng* **6**: 351-359.
- Reichert JC, Saifzadeh S, Wulschleger ME, Epari DR, Schutz MA, Duda GN, Schell H, van Griensven M, Redl H, Hutmacher DW (2009) The challenge of establishing preclinical models for segmental bone defect research. *Biomaterials* **30**: 2149-2163.
- Reichert JC, Quent VM, Noth U, Hutmacher DW (2011) Ovine cortical osteoblasts outperform bone marrow cells in an ectopic bone assay. *J Tissue Eng Regen Med* **5**: 831-844.
- Reichert JC, Cipitria A, Epari DR, Saifzadeh S, Krishnakanth P, Berner A, Woodruff MA, Schell H, Mehta M, Schuetz MA, Duda GN, Hutmacher DW (2012) A tissue engineering solution for segmental defect regeneration in load-bearing long bones. *Sci Transl Med* **4**: 141ra93.
- Ren Y, Liu B, Feng Y, Shu L, Cao X, Karaplis A, Goltzman D, Miao D (2011) Endogenous PTH deficiency impairs fracture healing and impedes the fracture-healing efficacy of exogenous PTH(1-34). *PLoS One* **6**: e23060.
- Reyes R, De la Riva B, Delgado A, Hernandez A, Sanchez E, Evora C (2012) Effect of triple growth factor controlled delivery by a brushite-PLGA system on a bone defect. *Injury* **43**: 334-342.
- Reynolds DG, Takahata M, Lerner AL, O'Keefe RJ, Schwarz EM, Awad HA (2011) Teriparatide therapy enhances devitalized femoral allograft osseointegration and biomechanics in a murine model. *Bone* **48**: 562-570.
- Riedel GE, Valentin-Opran A (1999) Clinical evaluation of rhBMP-2/ACS in orthopedic trauma: a progress report. *Orthopedics* **22**: 663-665.
- Rihani-Bisharat S, Maor G, Lewinson D (1998) *In vivo* anabolic effects of parathyroid hormone (PTH) 28-48 and N-terminal fragments of PTH and PTH-related protein on neonatal mouse bones. *Endocrinology* **139**: 974-981.
- Rimondini L, Nicoli-Aldini N, Fini M, Guzzardella G, Tschon M, Giardino R (2005) *In vivo* experimental study on bone regeneration in critical bone defects using an injectable biodegradable PLA/PGA copolymer. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **99**: 148-154.
- Ripamonti U, Van Den Heever B, Sampath TK, Tucker MM, Rueger DC, Reddi AH (1996) Complete regeneration of bone in the baboon by recombinant human osteogenic protein-1 (hOP-1, bone morphogenetic protein-7). *Growth Factors* **13**: 273-289.
- Ripamonti U, Duneas N, Van Den Heever B, Bosch C, Crooks J (1997) Recombinant transforming growth factor-beta1 induces endochondral bone in the baboon and synergizes with recombinant osteogenic protein-1 (bone morphogenetic protein-7) to initiate rapid bone formation. *J Bone Miner Res* **12**: 1584-1595.
- Ripamonti U, Van Den Heever B, Crooks J, Tucker MM, Sampath TK, Rueger DC, Reddi AH (2000) Long-term evaluation of bone formation by osteogenic protein 1 in the baboon and relative efficacy of bone-derived bone morphogenetic proteins delivered by irradiated xenogeneic collagenous matrices. *J Bone Miner Res* **15**: 1798-1809.
- Ripamonti U, Crooks J, Rueger DC (2001a) Induction of bone formation by recombinant human osteogenic protein-1 and sintered porous hydroxyapatite in adult primates. *Plast Reconstr Surg* **107**: 977-988.
- Ripamonti U, Ramoshebi LN, Matsaba T, Tasker J, Crooks J, Teare J (2001b) Bone induction by BMPs/OPs and related family members in primates. *J Bone Joint Surg Am* **83 Suppl 1**: S116-127.
- Ripamonti U, Ramoshebi LN, Teare J, Renton L, Ferretti C (2008) The induction of endochondral bone formation by transforming growth factor-beta(3): experimental studies in the non-human primate *Papio ursinus*. *J Cell Mol Med* **12**: 1029-1048.
- Ripamonti U, Ferretti C, Teare J, Blann L (2009a) Transforming growth factor-beta isoforms and the induction of bone formation: implications for reconstructive craniofacial surgery. *J Craniofac Surg* **20**: 1544-1555.
- Ripamonti U, Parak R, Petit JC (2009b) Induction of cementogenesis and periodontal ligament regeneration by recombinant human transforming growth factor-beta3 in Matrigel with rectus abdominis responding cells. *J Periodontal Res* **44**: 81-87.

- Ripamonti U, Klar RM, Renton LF, Ferretti C (2010) Synergistic induction of bone formation by hOP-1, hTGF- β 3 and inhibition by zoledronate in macroporous coral-derived hydroxyapatites. *Biomaterials* **31**: 6400-6410.
- Ristiniemi J, Flinkkila T, Hyvonen P, Lakovaara M, Pakarinen H, Jalovaara P (2007) RhBMP-7 accelerates the healing in distal tibial fractures treated by external fixation. *J Bone Joint Surg Br* **89**: 265-272.
- Rizk A, Rabie AB (2013) Human dental pulp stem cells expressing transforming growth factor β 3 transgene for cartilage-like tissue engineering. *Cytotherapy* **15**: 712-725.
- Roberts MD, Santner TJ, Hart RT (2009) Local bone formation due to combined mechanical loading and intermittent hPTH-(1-34) treatment and its correlation to mechanical signal distributions. *J Biomech* **42**: 2431-2438.
- Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH (2008) Mechanical stimulation of bone *in vivo* reduces osteocyte expression of Sost/sclerostin. *J Biol Chem* **283**: 5866-5875.
- Roldan JC, Jepsen S, Miller J, Freitag S, Rueger DC, Acil Y, Terheyden H (2004) Bone formation in the presence of platelet-rich plasma vs. bone morphogenetic protein-7. *Bone* **34**: 80-90.
- Roldan JC, Detsch R, Schaefer S, Chang E, Kelantan M, Waiss W, Reichert TE, Gurtner GC, Deisinger U (2010) Bone formation and degradation of a highly porous biphasic calcium phosphate ceramic in presence of BMP-7, VEGF and mesenchymal stem cells in an ectopic mouse model. *J Craniomaxillofac Surg* **38**: 423-430.
- Ronga M, Baldo F, Zappala G, Cherubino P (2006) Recombinant human bone morphogenetic protein-7 for treatment of long bone non-union: an observational, retrospective, non-randomized study of 105 patients. *Injury Suppl* **3**: S51-S56.
- Roosa SM, Kempainen JM, Moffitt EN, Krebsbach PH, Hollister SJ (2009) The pore size of polycaprolactone scaffolds has limited influence on bone regeneration in an *in vivo* model. *J Biomed Mater Res A* **92**: 359-368.
- Rowshan HH, Parham MA, Baur DA, McEntee RD, Cauley E, Carriere DT, Wood JC, Demers WJ, Pizarro JM (2010) Effect of intermittent systemic administration of recombinant parathyroid hormone (1-34) on mandibular fracture healing in rats. *J Oral Maxillofac Surg* **68**: 260-267.
- Rozen N, Lewinson D, Bick T, Jacob ZC, Stein H, Soudry M (2007) Fracture repair: modulation of fracture-callus and mechanical properties by sequential application of IL-6 following PTH 1-34 or PTH 28-48. *Bone* **41**: 437-445.
- Ryder KM, Tanner SB, Carbone L, Williams JE, Taylor HM, Bush A, Pintea V, Watsky MA (2010) Teriparatide is safe and effectively increases bone biomarkers in institutionalized individuals with osteoporosis. *J Bone Miner Metab* **28**: 233-239.
- Saito N, Okada T, Horiuchi H, Ota H, Takahashi J, Murakami N, Nawata M, Kojima S, Nozaki K, Takaoka K (2003) Local bone formation by injection of recombinant human bone morphogenetic protein-2 contained in polymer carriers. *Bone* **32**: 381-386.
- Salkeld SL, Patron LP, Barrack RL, Cook SD (2001) The effect of osteogenic protein-1 on the healing of segmental bone defects treated with autograft or allograft bone. *J Bone Joint Surg Am* **83**: 803-816.
- Samee M, Kasugai S, Kondo H, Ohya K, Shimokawa H, Kuroda S (2008) Bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) transfection to human periosteal cells enhances osteoblast differentiation and bone formation. *J Pharmacol Sci* **108**: 18-31.
- Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, White KH, Coughlin JE, Tucker MM, Pang RH (1992) Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation *in vivo* with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation *in vitro*. *J Biol Chem* **267**: 20352-20362.
- Sandhu HS, Kanim LE, Kabo JM, Toth JM, Zeegan EN, Liu D, Seeger LL, Dawson EG (1995) Evaluation of rhBMP-2 with an OPLA carrier in a canine posterolateral (transverse process) spinal fusion model. *Spine* **20**: 2669-2682.
- Santana RB, Trackman PC (2006) Controlled release of fibroblast growth factor 2 stimulates bone healing in an animal model of diabetes mellitus. *Int J Oral Maxillofac Implants* **21**: 711-718.
- Sarban S, Senkoylu A, Isikan UE, Korkusuz P, Korkusuz F (2009) Can rhBMP-2 containing collagen sponges enhance bone repair in ovariectomized rats?: a preliminary study. *Clin Orthop Relat Res* **467**: 3113-3120.
- Sawada Y, Hokugo A, Nishiura A, Hokugo R, Matsumoto N, Morita S, Tabata Y (2009) A trial of alveolar cleft bone regeneration by controlled release of bone morphogenetic protein: an experimental study in rabbits. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **108**: 812-820.
- Schafer AL, Burghardt AJ, Sellmeyer DE, Palermo L, Shoback DM, Majumdar S, Black DM (2013) Postmenopausal women treated with combination parathyroid hormone (1-84) and ibandronate demonstrate different microstructural changes at the radius vs. tibia: the PTH and Ibandronate Combination Study (PICS). *Osteoporos Int* **24**: 2591-2601.
- Schauss SM, Hinz M, Mayr E, Bach CM, Krismer M, Fischer M (2006) Inferior stability of a biodegradable cement plug. 122 total hip replacements randomized to degradable or non-degradable cement restrictor. *Arch Orthop Trauma Surg* **126**: 324-329.
- Schindeler A, Birke O, Yu NY, Morse A, Ruys A, Baldock PA, Little DG (2011) Distal tibial fracture repair in a neurofibromatosis type 1-deficient mouse treated with recombinant bone morphogenetic protein and a bisphosphonate. *J Bone Joint Surg Br* **93**: 1134-1139.
- Schmidmaier G, Wildemann B, Cromme F, Kandziora F, Haas NP, Raschke M (2002) Bone morphogenetic protein-2 coating of titanium implants increases biomechanical strength and accelerates bone remodeling in fracture treatment: a biomechanical and histological study in rats. *Bone* **30**: 816-822.
- Schopper C, Moser D, Spassova E, Goriwoda W, Lagogiannis G, Hoering B, Ewers R, Redl H (2008) Bone regeneration using a naturally grown HA/TCP carrier

loaded with rh BMP-2 is independent of barrier-membrane effects. *J Biomed Mater Res A* **85**: 954-963.

Schwarz C, Wulsten D, Ellinghaus A, Lienau J, Willie BM, Duda GN (2012) Mechanical load modulates the stimulatory effect of BMP2 in a rat nonunion model. *Tissue Eng Part A* **19**: 247-254.

Sciadini MF, Johnson KD (2000) Evaluation of recombinant human bone morphogenetic protein-2 as a bone-graft substitute in a canine segmental defect model. *J Orthop Res* **18**: 289-302.

Seeherman HJ, Boussein M, Kim H, Li R, Li XJ, Aiolo M, Wozney JM (2004) Recombinant human bone morphogenetic protein-2 delivered in an injectable calcium phosphate paste accelerates osteotomy-site healing in a nonhuman primate model. *J Bone Joint Surg Am* **86**: 1961-1972.

Semino CE (2008) Self-assembling peptides: from bio-inspired materials to bone regeneration. *J Dent Res* **87**: 606-616.

Sheehan JP, Sheehan JM, Seeherman H, Quigg M, Helm GA (2003) The safety and utility of recombinant human bone morphogenetic protein-2 for cranial procedures in a nonhuman primate model. *J Neurosurg* **98**: 125-130.

Shin H, Jo S, Mikos AG (2003) Biomimetic materials for tissue engineering. *Biomaterials* **24**: 4353-4364.

Shirakata Y, Taniyama K, Yoshimoto T, Miyamoto M, Takeuchi N, Matsuyama T, Noguchi K (2010) Regenerative effect of basic fibroblast growth factor on periodontal healing in two-wall intrabony defects in dogs. *J Clin Periodontol* **37**: 374-381.

Sigurdsson TJ, Lee MB, Kubota K, Turek TJ, Wozney JM, Wikesjo UM (1995) Periodontal repair in dogs: recombinant human bone morphogenetic protein-2 significantly enhances periodontal regeneration. *J Periodontol* **66**: 131-138.

Sigurdsson TJ, Nygaard L, Tatakis DN, Fu E, Turek TJ, Jin L, Wozney JM, Wikesjo UM (1996) Periodontal repair in dogs: evaluation of rhBMP-2 carriers. *Int J Periodontics Restorative Dent* **16**: 524-537.

Sigurdsson TJ, Fu E, Tatakis DN, Rohrer MD, Wikesjo UM (1997) Bone morphogenetic protein-2 for peri-implant bone regeneration and osseointegration. *Clin Oral Implants Res* **8**: 367-374.

Simmons CA, Alsberg E, Hsiong S, Kim WJ, Mooney DJ (2004) Dual growth factor delivery and controlled scaffold degradation enhance *in vivo* bone formation by transplanted bone marrow stromal cells. *Bone* **35**: 562-569.

Singh K, Smucker JD, Gill S, Boden SD (2006) Use of recombinant human bone morphogenetic protein-2 as an adjunct in posterolateral lumbar spine fusion: a prospective CT-scan analysis at one and two years. *J Spinal Disord Tech* **19**: 416-423.

Skurla CP, James SP (2005) Assessing the dog as a model for human total hip replacement: analysis of 38 postmortem-retrieved canine cemented acetabular components. *J Biomed Mater Res B Appl Biomater* **73**: 260-270.

Sloan AV, Martin JR, Li S, Li J (2010) Parathyroid hormone and bisphosphonate have opposite effects on stress fracture repair. *Bone* **47**: 235-240.

Smith EL, Kanczler JM, Roberts CA, Oreffo RO (2012) Developmental cues for bone formation from parathyroid hormone and parathyroid hormone-related protein in an *ex vivo* organotypic culture system of embryonic chick femora. *Tissue Eng Part C Methods* **18**: 984-994.

Smith EL, Kanczler JM, Oreffo RO (2013) A new take on an old story: chick limb organ culture for skeletal niche development and regenerative medicine evaluation. *Eur Cell Mater* **26**: 91-106.

Smith EL, Kanczler JM, Gothard D, Roberts CA, Wells JA, White LJ, Qutachi O, Sawkins MJ, Peto H, Rashidi H, Rojo L, Stevens MM, El Haj AJ, Rose FR, Shakesheff KM, Oreffo RO (2014a) Evaluation of skeletal tissue repair, Part 2: Enhancement of skeletal tissue repair through dual-growth-factor-releasing hydrogels within an *ex vivo* chick femur defect model. *Acta Biomater* doi: 10.1016/j.actbio.2014.05.025 [Epub ahead of print].

Smith EL, Kanczler JM, Gothard D, Roberts CA, Wells JA, White LJ, Qutachi O, Sawkins MJ, Peto H, Rashidi H, Rojo L, Stevens MM, El Haj AJ, Rose FR, Shakesheff KM, Oreffo RO (2014b) Evaluation of skeletal tissue repair. Part 1. Assessment of novel growth factor-releasing hydrogels in an *ex vivo* chick femur defect model. *Acta Biomater* doi: 10.1016/j.actbio.2014.06.011 [Epub ahead of print].

Soballe K, Jensen TB, Mouzin O, Kidder L, Bechtold JE (2004) Differential effect of a bone morphogenetic protein-7 (OP-1) on primary and revision loaded, stable implants with allograft. *J Biomed Mater Res A* **71**: 569-576.

Southwood LL, Frisbie DD, Kawcak CE, Ghivizzani SC, Evans CH, McIlwraith CW (2004) Evaluation of Ad-BMP-2 for enhancing fracture healing in an infected defect fracture rabbit model. *J Orthop Res* **22**: 66-72.

Southwood LL, Kawcak CE, Hidaka C, McIlwraith CW, Werpy N, Macleay J, Frisbie DD (2012) Evaluation of direct *in vivo* gene transfer in an equine metacarpal IV ostectomy model using an adenoviral vector encoding the bone morphogenetic protein-2 and protein-7 gene. *Vet Surg* **41**: 345-354.

Spiro AS, Beil FT, Baranowsky A, Barvencik F, Schilling AF, Nguyen K, Khadem S, Seitz S, Rueger JM, Schinke T, Amling M (2010) BMP-7-induced ectopic bone formation and fracture healing is impaired by systemic NSAID application in C57BL/6-mice. *J Orthop Res* **28**: 785-791.

Springer IN, Niehoff P, Acil Y, Marget M, Lange A, Warnke PH, Pielenz H, Roldan JC, Wiltfang J (2008) BMP-2 and bFGF in an irradiated bone model. *J Craniomaxillofac Surg* **36**: 210-217.

Srouji S, Ben-David D, Lotan R, Livne E, Avrahami R, Zussman E (2011) Slow-release human recombinant bone morphogenetic protein-2 embedded within electrospun scaffolds for regeneration of bone defect: *in vitro* and *in vivo* evaluation. *Tissue Eng Part A* **17**: 269-277.

Steinhardt Y, Aslan H, Regev E, Zilberman Y, Kallai I, Gazit D, Gazit Z (2008) Maxillofacial-derived stem cells regenerate critical mandibular bone defect. *Tissue Eng Part A* **14**: 1763-1773.

Stephan SJ, Tholpady SS, Gross B, Petrie-Aronin CE, Botchway EA, Nair LS, Ogle RC, Park SS (2010) Injectable tissue-engineered bone repair of a rat calvarial defect. *Laryngoscope* **120**: 895-901.

- Stewart AF, Cain RL, Burr DB, Jacob D, Turner CH, Hock JM (2000) Six-month daily administration of parathyroid hormone and parathyroid hormone-related protein peptides to adult ovariectomized rats markedly enhances bone mass and biomechanical properties: a comparison of human parathyroid hormone 1-34, parathyroid hormone-related protein 1-36, and SDZ-parathyroid hormone 893. *J Bone Miner Res* **15**: 1517-1525.
- Strom O, Jonsson B, Kanis JA (2013) Intervention thresholds for denosumab in the UK using a FRAX(R)-based cost-effectiveness analysis. *Osteoporos Int* **24**: 1491-1502.
- Sumner DR, Turner TM, Urban RM, Turek T, Seeherman H, Wozney JM (2004) Locally delivered rhBMP-2 enhances bone ingrowth and gap healing in a canine model. *J Orthop Res* **22**: 58-65.
- Sun H, Jung Y, Shiozawa Y, Taichman RS, Krebsbach PH (2012a) Erythropoietin modulates the structure of bone morphogenetic protein 2-engineered cranial bone. *Tissue Eng Part A* **18**: 2095-2105.
- Sun P, Wang J, Zheng Y, Fan Y, Gu Z (2012b) BMP2/7 heterodimer is a stronger inducer of bone regeneration in peri-implant bone defects model than BMP2 or BMP7 homodimer. *Dent Mater J* **31**: 239-248.
- Sundelacruz S, Kaplan DL (2009) Stem cell- and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. *Semin Cell Dev Biol* **20**: 646-655.
- Suzuki Y, Tanihara M, Suzuki K, Saitou A, Sufan W, Nishimura Y (2000) Alginate hydrogel linked with synthetic oligopeptide derived from BMP-2 allows ectopic osteoinduction *in vivo*. *J Biomed Mater Res* **50**: 405-409.
- Swionkowski MF, Aro HT, Donell S, Esterhai JL, Goulet J, Jones A, Kregor PJ, Nordsletten L, Paiement G, Patel A (2006) Recombinant human bone morphogenetic protein-2 in open tibial fractures. A subgroup analysis of data combined from two prospective randomized studies. *J Bone Joint Surg Am* **88**: 1258-1265.
- Tabata Y (2003) Tissue regeneration based on growth factor release. *Tissue Eng* **9 Suppl 1**: S5-15.
- Taghavi CE, Lee KB, Keorochana G, Tzeng ST, Yoo JH, Wang JC (2010) Bone morphogenetic protein-2 and bone marrow aspirate with allograft as alternatives to autograft in instrumented revision posterolateral lumbar spinal fusion: a minimum two-year follow-up study. *Spine* **35**: 1144-1150.
- Tagil M, Jeppsson C, Wang JS, Aspenberg P (2003) No augmentation of morselized and impacted bone graft by OP-1 in a weight-bearing model. *Acta Orthop Scand* **74**: 742-748.
- Tagil M, McDonald MM, Morse A, Peacock L, Mikulec K, Amanat N, Godfrey C, Little DG (2010) Intermittent PTH(1-34) does not increase union rates in open rat femoral fractures and exhibits attenuated anabolic effects compared to closed fractures. *Bone* **46**: 852-859.
- Takahata M, Schwarz EM, Chen T, O'Keefe RJ, Awad HA (2012) Delayed short-course treatment with teriparatide (PTH(1-34)) improves femoral allograft healing by enhancing intramembranous bone formation at the graft-host junction. *J Bone Miner Res* **27**: 26-37.
- Takayama S, Murakami S, Shimabukuro Y, Kitamura M, Okada H (2001) Periodontal regeneration by FGF-2 (bFGF) in primate models. *J Dent Res* **80**: 2075-2079.
- Takechi M, Tatehara S, Satomura K, Fujisawa K, Nagayama M (2008) Effect of FGF-2 and melatonin on implant bone healing: a histomorphometric study. *J Mater Sci Mater Med* **19**: 2949-2952.
- Takigami H, Kumagai K, Latson L, Togawa D, Bauer T, Powell K, Butler RS, Muschler GF (2007) Bone formation following OP-1 implantation is improved by addition of autogenous bone marrow cells in a canine femur defect model. *J Orthop Res* **25**: 1333-1342.
- Takimoto Y, Dixit V, Arthur M, Gitnick G (2003) *De novo* liver tissue formation in rats using a novel collagen-polypropylene scaffold. *Cell Transplant* **12**: 413-421.
- Tan Z, Zhao Q, Gong P, Wu Y, Wei N, Yuan Q, Wang C, Liao D, Tang H (2009) Research on promoting periodontal regeneration with human basic fibroblast growth factor-modified bone marrow mesenchymal stromal cell gene therapy. *Cytotherapy* **11**: 317-325.
- Tang QO, Shakib K, Heliotis M, Tsiridis E, Mantalaris A, Ripamonti U (2009) TGF-beta3: A potential biological therapy for enhancing chondrogenesis. *Expert Opin Biol Ther* **9**: 689-701.
- Teare JA, Ramoshebi LN, Ripamonti U (2008) Periodontal tissue regeneration by recombinant human transforming growth factor-beta 3 in *Papio ursinus*. *J Periodontol Res* **43**: 1-8.
- Terella A, Mariner P, Brown N, Anseth K, Streubel SO (2010) Repair of a calvarial defect with biofactor and stem cell-embedded polyethylene glycol scaffold. *Arch Facial Plast Surg* **12**: 166-171.
- Terheyden H, Warnke P, Dunsche A, Jepsen S, Brenner W, Palmie S, Toth C, Rueger DR (2001) Mandibular reconstruction with prefabricated vascularized bone grafts using recombinant human osteogenic protein-1: an experimental study in miniature pigs. Part II: transplantation. *Int J Oral Maxillofac Surg* **30**: 469-478.
- Thorey F, Menzel H, Lorenz C, Gross G, Hoffmann A, Windhagen H (2011) Osseointegration by bone morphogenetic protein-2 and transforming growth factor beta2 coated titanium implants in femora of New Zealand white rabbits. *Indian J Orthop* **45**: 57-62.
- Thorwarth M, Schultze-Mosgau S, Kessler P, Wiltfang J, Schlegel KA (2005) Bone regeneration in osseous defects using a resorbable nanoparticulate hydroxyapatite. *J Oral Maxillofac Surg* **63**: 1626-1633.
- Tolli H, Kujala S, Jamsa T, Jalovaara P (2011) Reindeer bone extract can heal the critical-size rat femur defect. *Int Orthop* **35**: 615-622.
- Trejo CG, Lozano D, Manzano M, Doadrio JC, Salinas AJ, Dapia S, Gomez-Barrena E, Vallet-Regi M, Garcia-Hondurilla N, Bujan J, Esbrit P (2010) The osteoinductive properties of mesoporous silicate coated with osteostatin in a rabbit femur cavity defect model. *Biomaterials* **31**: 8564-8573.
- Tressler MA, Richards JE, Sofianos D, Comrie FK, Kregor PJ, Obrebsky WT (2011) Bone morphogenetic protein-2 compared to autologous iliac crest bone graft in the treatment of long bone nonunion. *Orthopedics* **34**: e877-884.

- Triplet RG, Nevins M, Marx RE, Spagnoli DB, Oates TW, Moy PK, Boyne PJ (2009) Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. *J Oral Maxillofac Surg* **67**: 1947-1960.
- Tsiridis E, Ali Z, Bhalla A, Heliotis M, Gurav N, Deb S, DiSilvio L (2007a) *In vitro* and *in vivo* optimization of impaction allografting by demineralization and addition of rh-OP-1. *J Orthop Res* **25**: 1425-1437.
- Tsiridis E, Morgan EF, Bancroft JM, Song M, Kain M, Gerstenfeld L, Einhorn TA, Bouxsein ML, Tornetta P, 3rd (2007b) Effects of OP-1 and PTH in a new experimental model for the study of metaphyseal bone healing. *J Orthop Res* **25**: 1193-1203.
- Tsuchie H, Miyakoshi N, Kasukawa Y, Aonuma H, Shimada Y (2013) Intermittent administration of human parathyroid hormone before osteosynthesis stimulates cancellous bone union in ovariectomized rats. *Tohoku J Exp Med* **229**: 19-28.
- Tsurushima H, Marushima A, Suzuki K, Oyane A, Sogo Y, Nakamura K, Matsumura A, Ito A (2010) Enhanced bone formation using hydroxyapatite ceramic coated with fibroblast growth factor-2. *Acta Biomater* **6**: 2751-2759.
- Tsuzuki N, Otsuka K, Seo J, Yamada K, Haneda S, Furuoka H, Tabata Y, Sasaki N (2012) *In vivo* osteoinductivity of gelatin beta-tri-calcium phosphate sponge and bone morphogenetic protein-2 on an equine third metacarpal bone defect. *Res Vet Sci* **93**: 1021-1025.
- Turgeman G, Pittman DD, Muller R, Kurkalli BG, Zhou S, Pelled G, Peyser A, Zilberman Y, Moutsatsos IK, Gazit D (2001) Engineered human mesenchymal stem cells: a novel platform for skeletal cell mediated gene therapy. *J Gene Med* **3**: 240-251.
- Turner NJ, Keane TJ, Badylak SF (2013) Lessons from developmental biology for regenerative medicine. *Birth Defects Res C Embryo Today* **99**: 149-159.
- Ueki K, Takazakura D, Marukawa K, Shimada M, Nakagawa K, Takatsuka S, Yamamoto E (2003) The use of polylactic acid/polyglycolic acid copolymer and gelatin sponge complex containing human recombinant bone morphogenetic protein-2 following condylectomy in rabbits. *J Craniomaxillofac Surg* **31**: 107-114.
- Urist MR (1965) Bone: formation by autoinduction. *Science* **150**: 893-899.
- Urist MR, Strates BS (1971) Bone morphogenetic protein. *J Dental Res* **50**: 1392-1406.
- Vaccaro AR, Patel T, Fischgrund J, Anderson DG, Truumees E, Herkowitz H, Phillips F, Hilibrand A, Albert TJ (2003) A pilot safety and efficacy study of OP-1 putty (rhBMP-7) as an adjunct to iliac crest autograft in posterolateral lumbar fusions. *Eur Spine J* **12**: 495-500.
- Vaccaro AR, Patel T, Fischgrund J, Anderson DG, Truumees E, Herkowitz HN, Phillips F, Hilibrand A, Albert TJ, Wetzel T, McCulloch JA (2004) A pilot study evaluating the safety and efficacy of OP-1 Putty (rhBMP-7) as a replacement for iliac crest autograft in posterolateral lumbar arthrodesis for degenerative spondylolisthesis. *Spine* **29**: 1885-1892.
- Vaccaro AR, Patel T, Fischgrund J, Anderson DG, Truumees E, Herkowitz H, Phillips F, Hilibrand A, Albert TJ (2005) A 2-year follow-up pilot study evaluating the safety and efficacy of op-1 putty (rhbmp-7) as an adjunct to iliac crest autograft in posterolateral lumbar fusions. *Eur Spine J* **14**: 623-629.
- Vahle JL, Long GG, Sandusky G, Westmore M, Ma YL, Sato M (2004) Bone neoplasms in F344 rats given teriparatide [rhPTH(1-34)] are dependent on duration of treatment and dose. *Toxicol Pathol* **32**: 426-438.
- Vahle JL, Zuehlke U, Schmidt A, Westmore M, Chen P, Sato M (2008) Lack of bone neoplasms and persistence of bone efficacy in cynomolgus macaques after long-term treatment with teriparatide [rhPTH(1-34)]. *J Bone Miner Res* **23**: 2033-2039.
- van Amerongen R, Fuerer C, Mizutani M, Nusse R (2012) Wnt5a can both activate and repress Wnt/beta-catenin signaling during mouse embryonic development. *Dev Biol* **369**: 101-114.
- van den Bergh JP, ten Bruggenkate CM, Groeneveld HH, Burger EH, Tuinzing DB (2000) Recombinant human bone morphogenetic protein-7 in maxillary sinus floor elevation surgery in 3 patients compared to autogenous bone grafts. A clinical pilot study. *J Clin Periodontol* **27**: 627-636.
- van der Stok J, Wang H, Amin Yavari S, Siebelt M, Sandker M, Waarsing JH, Verhaar JA, Jahr H, Zadpoor AA, Leeuwenburgh SC, Weinans H (2013) Enhanced bone regeneration of cortical segmental bone defects using porous titanium scaffolds incorporated with colloidal gelatin gels for time- and dose-controlled delivery of dual growth factors. *Tissue Eng Part A* **19**: 2605-2614.
- van Staa TP, Dennison EM, Leufkens HG, Cooper C (2001) Epidemiology of fractures in England and Wales. *Bone* **29**: 517-522.
- Vickery BH, Avnur Z, Cheng Y, Chiou SS, Leaffer D, Caulfield JP, Kimmel DB, Ho T, Krstenansky JL (1996) RS-66271, a C-terminally substituted analog of human parathyroid hormone-related protein (1-34), increases trabecular and cortical bone in ovariectomized, osteopenic rats. *J Bone Miner Res* **11**: 1943-1951.
- Vogt S, Wexel G, Tischer T, Schillinger U, Ueblicher P, Wagner B, Hensler D, Wilisch J, Geis C, Wubbenhorst D, Aigner J, Gerg M, Kruger A, Salzmann GM, Martinek V, Anton M, Plank C, Imhoff AB, Gansbacher B (2009) The influence of the stable expression of BMP2 in fibrin clots on the remodelling and repair of osteochondral defects. *Biomaterials* **30**: 2385-2392.
- Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, Israel DI, Hewick RM, Kerns KM, LaPan P (1990) Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci USA* **87**: 2220-2224.
- Wang R, Zou Y, Yuan Z, Wang Y, Chen Y, Mao Y, Zhu ZA, Li H, Tang X, Lu J, Yi J (2009) Autografts and xenografts of skin fibroblasts delivering BMP-2 effectively promote orthotopic and ectopic osteogenesis. *Anat Rec* **292**: 777-786.
- Wang J, Zheng Y, Zhao J, Liu T, Gao L, Gu Z, Wu G (2012) Low-dose rhBMP2/7 heterodimer to reconstruct peri-implant bone defects: a micro-CT evaluation. *J Clin Periodontol* **39**: 98-105.

- Warnke PH, Springer IN, Acil Y, Julga G, Wiltfang J, Ludwig K, Russo PA, Sherry E, Sivananthan S, Hedderich J, Terheyden H (2006) The mechanical integrity of *in vivo* engineered heterotopic bone. *Biomaterials* **27**: 1081-1087.
- Wei G, Jin Q, Giannobile WV, Ma PX (2007) The enhancement of osteogenesis by nano-fibrous scaffolds incorporating rhBMP-7 nanospheres. *Biomaterials* **28**: 2087-2096.
- Weir EC, Terwilliger G, Sartori L, Insogna KL (1992) Synthetic parathyroid hormone-like protein (1-74) is anabolic for bone *in vivo*. *Calcif Tissue Int* **51**: 30-34.
- Welch RD, Jones AL, Bucholz RW, Reinert CM, Tjia JS, Pierce WA, Wozney JM, Li XJ (1998) Effect of recombinant human bone morphogenetic protein-2 on fracture healing in a goat tibial fracture model. *J Bone Miner Res* **13**: 1483-1490.
- Wernike E, Montjovent MO, Liu Y, Wismeijer D, Hunziker EB, Siebenrock KA, Hofstetter W, Klenke FM (2010) VEGF incorporated into calcium phosphate ceramics promotes vascularisation and bone formation *in vivo*. *Eur Cell Mater* **19**: 30-40.
- Whang K, Tsai DC, Nam EK, Aitken M, Sprague SM, Patel PK, Healy KE (1998) Ectopic bone formation *via* rhBMP-2 delivery from porous bioabsorbable polymer scaffolds. *J Biomed Mater Res* **42**: 491-499.
- Wikesjo UM, Qahash M, Polimeni G, Susin C, Shanaman RH, Rohrer MD, Wozney JM, Hall J (2008) Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: histologic observations. *J Clin Periodontol* **35**: 1001-1010.
- Wolf M, Lossdorfer S, Abuduwali N, Meyer R, Kebir S, Gotz W, Jager A (2012) Effect of intermittent PTH(1-34) on human periodontal ligament cells transplanted into immunocompromised mice. *Tissue Eng Part A* **18**: 1849-1856.
- Woo BH, Fink BF, Page R, Schrier JA, Jo YW, Jiang G, DeLuca M, Vasconez HC, DeLuca PP (2001) Enhancement of bone growth by sustained delivery of recombinant human bone morphogenetic protein-2 in a polymeric matrix. *Pharm Res* **18**: 1747-1753.
- Xiao C, Zhou H, Ge S, Tang T, Hou H, Luo M, Fan X (2010) Repair of orbital wall defects using biocoral scaffolds combined with bone marrow stem cells enhanced by human bone morphogenetic protein-2 in a canine model. *Int J Mol Med* **26**: 517-525.
- Xiao C, Zhou H, Liu G, Zhang P, Fu Y, Gu P, Hou H, Tang T, Fan X (2011) Bone marrow stromal cells with a combined expression of BMP-2 and VEGF-165 enhanced bone regeneration. *Biomed Mater* **6**: 015013.
- Xu L, Lv K, Zhang W, Zhang X, Jiang X, Zhang F (2012) The healing of critical-size calvarial bone defects in rat with rhPDGF-BB, BMSCs, and beta-TCP scaffolds. *J Mater Sci Mater Med* **23**: 1073-1084.
- Xue Y, Zhang Z, Karaplis AC, Hendy GN, Goltzman D, Miao D (2005) Exogenous PTH-related protein and PTH improve mineral and skeletal status in 25-hydroxyvitamin D-1 α -hydroxylase and PTH double knockout mice. *J Bone Miner Res* **20**: 1766-1777.
- Yang X, Tare RS, Partridge KA, Roach HI, Clarke NM, Howdle SM, Shakesheff KM, Oreffo RO (2003) Induction of human osteoprogenitor chemotaxis, proliferation, differentiation, and bone formation by osteoblast stimulating factor-1/pleiotrophin: osteoconductive biomimetic scaffolds for tissue engineering. *J Bone Miner Res* **18**: 47-57.
- Yang M, Ma QJ, Dang GT, Ma K, Chen P, Zhou CY (2005) *In vitro* and *in vivo* induction of bone formation based on *ex vivo* gene therapy using rat adipose-derived adult stem cells expressing BMP-7. *Cytotherapy* **7**: 273-281.
- Yang L, Zhang Y, Dong R, Peng L, Liu X, Wang Y, Cheng X (2010) Effects of adenoviral-mediated coexpression of bone morphogenetic protein-7 and insulin-like growth factor-1 on human periodontal ligament cells. *J Periodontol Res* **45**: 532-540.
- Yang X, Han G, Pang X, Fan M (2012) Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation *in vitro* and *in vivo*. *J Biomed Mater Res A* doi: 10.1002/jbm.a.34064 [Epub ahead of print].
- Yang X, Muthukumaran P, DasDe S, Teoh SH, Choi H, Lim SK, Lee T (2013) Positive alterations of viscoelastic and geometric properties in ovariectomized rat femurs with concurrent administration of ibandronate and PTH. *Bone* **52**: 308-317.
- Yasko AW, Lane JM, Fellingner EJ, Rosen V, Wozney JM, Wang EA (1992) The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2). A radiographic, histological, and biomechanical study in rats. *J Bone Joint Surg Am* **74**: 659-670.
- Yazici C, Takahata M, Reynolds DG, Xie C, Samulski RJ, Samulski J, Beecham EJ, Gertzman AA, Spilker M, Zhang X, O'Keefe RJ, Awad HA, Schwarz EM (2011) Self-complementary AAV2.5-BMP2-coated femoral allografts mediated superior bone healing *versus* live autografts in mice with equivalent biomechanics to unfractured femur. *Mol Ther* **19**: 1416-1425.
- Yokota S, Sonohara S, Yoshida M, Murai M, Shimokawa S, Fujimoto R, Fukushima S, Kokubo S, Nozaki K, Takahashi K, Uchida T, Yokohama S, Sonobe T (2001) A new recombinant human bone morphogenetic protein-2 carrier for bone regeneration. *Int J Pharm* **223**: 69-79.
- Young S, Patel ZS, Kretlow JD, Murphy MB, Mountziaris PM, Baggett LS, Ueda H, Tabata Y, Jansen JA, Wong M, Mikos AG (2009) Dose effect of dual delivery of vascular endothelial growth factor and bone morphogenetic protein-2 on bone regeneration in a rat critical-size defect model. *Tissue Eng Part A* **15**: 2347-2362.
- Yu NY, Schindeler A, Peacock L, Mikulec K, Baldock PA, Ruys AJ, Little DG (2010a) *In vivo* local co-delivery of recombinant human bone morphogenetic protein-7 and pamidronate *via* poly-D, L-lactic acid. *Eur Cell Mater* **20**: 431-441.
- Yu YY, Lieu S, Lu C, Colnot C (2010b) Bone morphogenetic protein 2 stimulates endochondral ossification by regulating periosteal cell fate during bone repair. *Bone* **47**: 65-73.
- Yu B, Zhao X, Yang C, Crane J, Xian L, Lu W, Wan M, Cao X (2012a) Parathyroid hormone induces differentiation of mesenchymal stromal/stem cells by

enhancing bone morphogenetic protein signaling. *J Bone Miner Res* **27**: 2001-2014.

Yu X, Wang L, Jiang X, Rowe D, Wei M (2012b) Biomimetic CaP coating incorporated with parathyroid hormone improves the osseointegration of titanium implant. *J Mater Sci Mater Med* **23**: 2177-2186.

Yudell RM, Block MS (2000) Bone gap healing in the dog using recombinant human bone morphogenetic protein-2. *J Oral Maxillofac Surg* **58**: 761-766.

Yun JI, Wikesjo UM, Borke JL, Bisch FC, Lewis JE, Herold RW, Swiec GD, Wood JC, McPherson JC, 3rd (2010) Effect of systemic parathyroid hormone (1-34) and a beta-tricalcium phosphate biomaterial on local bone formation in a critical-size rat calvarial defect model. *J Clin Periodontol* **37**: 419-426.

Zabka AG, Pluhar GE, Edwards RB, 3rd, Manley PA, Hayashi K, Heiner JP, Kalscheur VL, Seeherman HJ, Markel (2001) Histomorphometric description of allograft bone remodeling and union in a canine segmental femoral defect model: a comparison of rhBMP-2, cancellous bone graft, and absorbable collagen sponge. *J Orthop Res* **19**: 318-327.

Zachos T, Diggs A, Weisbrode S, Bartlett J, Bertone A (2007) Mesenchymal stem cell-mediated gene delivery of bone morphogenetic protein-2 in an articular fracture model. *Mol Ther* **15**: 1543-1550.

Zakhary K, Motakis D, Hamdy RH, Campisi P, Amar Y, Lessard ML (2005) Effect of recombinant human bone morphogenetic protein 7 on bone density during distraction osteogenesis of the rabbit mandible. *J Otolaryngol* **34**: 407-414.

Zellin G, Linde A (2000) Effects of recombinant human fibroblast growth factor-2 on osteogenic cell populations during orthopedic osteogenesis *in vivo*. *Bone* **26**: 161-168.

Zhang R, An Y, Toth CA, Draughn RA, Dimaano NM, Hawkins MV (2004) Osteogenic protein-1 enhances osseointegration of titanium implants coated with periapatite in rabbit femoral defect. *J Biomed Mater Res B Appl Biomater* **71**: 408-413.

Zhang Y, Song J, Shi B, Wang Y, Chen X, Huang C, Yang X, Xu D, Cheng X (2007) Combination of scaffold and adenovirus vectors expressing bone morphogenetic protein-7 for alveolar bone regeneration at dental implant defects. *Biomaterials* **28**: 4635-4642.

Zhang Q, Cuartas E, Mehta N, Gilligan J, Ke HZ, Saltzman WM, Kotas M, Ma M, Rajan S, Chalouni C, Carlson J, Vignery A (2008) Replacement of bone marrow by bone in rat femurs: the bone bioreactor. *Tissue Eng Part A* **14**: 237-246.

Zhang J, Wang M, Cha JM, Mantalaris A (2009a) The incorporation of 70s bioactive glass to the osteogenic differentiation of murine embryonic stem cells in 3D bioreactors. *J Tissue Eng Regen Med* **3**: 63-71.

Zhang Y, Shi B, Li C, Wang Y, Chen Y, Zhang W, Luo T, Cheng X (2009b) The synergetic bone-forming effects of combinations of growth factors expressed by adenovirus vectors on chitosan/collagen scaffolds. *J Control Release* **136**: 172-178.

Zhang C, Wang KZ, Qiang H, Tang YL, Li Q, Li M, Dang XQ (2010a) Angiogenesis and bone regeneration via co-expression of the hVEGF and hBMP genes from

an adeno-associated viral vector *in vitro* and *in vivo*. *Acta Pharmacol Sin* **31**: 821-830.

Zhang Q, Carlson J, Ke HZ, Li J, Kim M, Murphy K, Mehta N, Gilligan J, Vignery A (2010b) Dramatic increase in cortical thickness induced by femoral marrow ablation followed by a 3-month treatment with PTH in rats. *J Bone Miner Res* **25**: 1350-1359.

Zhang Y, Fan W, Ma Z, Wu C, Fang W, Liu G, Xiao Y (2010c) The effects of pore architecture in silk fibroin scaffolds on the growth and differentiation of mesenchymal stem cells expressing BMP7. *Acta Biomater* **6**: 3021-3028.

Zhang W, Wang X, Wang S, Zhao J, Xu L, Zhu C, Zeng D, Chen J, Zhang Z, Kaplan DL, Jiang X (2011a) The use of injectable sonication-induced silk hydrogel for VEGF(165) and BMP-2 delivery for elevation of the maxillary sinus floor. *Biomaterials* **32**: 9415-9424.

Zhang Y, Fan W, Nothdurft L, Wu C, Zhou Y, Crawford R, Xiao Y (2011b) *In vitro* and *in vivo* evaluation of adenovirus combined silk fibroin scaffolds for bone morphogenetic protein-7 gene delivery. *Tissue Eng Part C Methods* **17**: 789-797.

Zhang Y, Cheng N, Miron R, Shi B, Cheng X (2012a) Delivery of PDGF-B and BMP-7 by mesoporous bioglass/silk fibrin scaffolds for the repair of osteoporotic defects. *Biomaterials* **33**: 6698-6708.

Zhang Y, Wu C, Luo T, Li S, Cheng X, Miron RJ (2012b) Synthesis and inflammatory response of a novel silk fibroin scaffold containing BMP7 adenovirus for bone regeneration. *Bone* **51**: 704-713.

Zhao J, Shen G, Liu C, Wang S, Zhang W, Zhang X, Ye D, Wei J, Zhang Z, Jiang X (2012) Enhanced healing of rat calvarial defects with sulfated chitosan-coated calcium-deficient hydroxyapatite/bone morphogenetic protein 2 scaffolds. *Tissue Eng Part A* **18**: 185-197.

Zhou H, Mak W, Kalak R, Street J, Fong-Yee C, Zheng Y, Dunstan CR, Seibel MJ (2009) Glucocorticoid-dependent Wnt signaling by mature osteoblasts is a key regulator of cranial skeletal development in mice. *Development* **136**: 427-436.

Zhu W, Rawlins BA, Boachie-Adjei O, Myers ER, Arimizu J, Choi E, Lieberman JR, Crystal RG, Hidaka C (2004) Combined bone morphogenetic protein-2 and -7 gene transfer enhances osteoblastic differentiation and spine fusion in a rodent model. *J Bone Miner Res* **19**: 2021-2032.

Zhu L, Chuanchang D, Wei L, Yilin C, Jiasheng D (2010) Enhanced healing of goat femur-defect using BMP7 gene-modified BMSCs and load-bearing tissue-engineered bone. *J Orthop Res* **28**: 412-418.

Zimmermann G, Wagner C, Schmeckenbecher K, Wentzensen A, Moghaddam A (2009) Treatment of tibial shaft non-unions: bone morphogenetic proteins *versus* autologous bone graft. *Injury* **40 Suppl 3**: S50-53.

Discussion with Reviewers

Reviewer I: Can biomaterials/scaffold influence the capability of stem cells to promote bone repair by themselves, without the use of growth factors? If yes,

through which molecular mechanisms (biomechanics/activation of particular signalling cascades)?

Authors: Biomaterials are discussed briefly within the introductory section, to equip the reader with a basic understanding of current tissue engineering strategies and thereby position them better to understand the tables. Biomaterials were not the aim of this review, and further discussion of the biomaterials may be misleading as the cohort of studies are not representative of the field as they were negated from the original search parameters. References to mesenchymal stem cells are only made when specifically discussing individual publications that utilised them and other cells. However, we have added our recent review to provide a reference overview for the reader.

Again, further discussion would not be representative of the entire field.

Reviewer I: Many of the biomaterials listed are used to deliver and release growth factors both in bone and articular cartilage. Did anyone study whether a specific biomaterial can influence in different ways the activity of the same growth factor in these tissues?

Authors: To cross compare all parameters within the data tables is a significant undertaking and would increase the overall review size extensively. However, the data are present in all supplementary tables for the reader to assess as required. Comments have been made throughout the text, highlighting these differences between studies and that interpretation should be carefully considered.