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Review Article

Delivery systems for bone growth factors — the new players in skeletal regeneration

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Abstract

Given the challenge of an increasing elderly population, the ability to repair and regenerate traumatised or lost tissue is a major clinical and socio-economic need. Pivotal in this process will be the ability to deliver appropriate growth factors in the repair cascade in a temporal and tightly regulated sequence using appropriately designed matrices and release technologies within a tissue engineering strategy. This review outlines the current concepts and challenges in growth factor delivery for skeletal regeneration and the potential of novel delivery matrices and biotechnologies to influence the healthcare of an increasing ageing population.

Introduction

"Will part for lack of nutriment – thy bones will wither in few years and vanish so" John Keats, the fall of Hyperion Canto 1, 1.11

The demographic challenge of an ageing population, in association with bone tissue loss as a consequence of the natural ageing process or from trauma or degenerative bone disease, has led to the need for procedures to create cartilage and bone for a variety of orthopaedic applications (Rose & Oreffo 2002). Tissue engineering aims to provide therapies to replace such damaged and diseased tissues by guiding and controlling tissue growth, either in-vitro or in-vivo, using a combination of biomaterials, cells and growth factors (Langer & Vacanti 1993, Sittinger et al 1996). With respect to skeletal tissue engineering, delivery of appropriate growth factors at specific times of bone regeneration, in combination with a scaffold conductive to cell attachment and function, and a rich source of precursor cells to augment and promote bone formation are essential (Zellin & Linde 1997; Peter et al 1998; Zellin 1998; Oreffo & Triffitt 1999; Vacanti 2000; Pearson et al 2002). Pivotal to the specific delivery of growth factors to the wound site is a delivery system or carrier material that will prevent the rapid diffusion of the growth factor, peptide or drug. To date, a plethora of delivery systems have been developed and modified, including collagen matrices, synthetic biodegradable polymers, bone graft substitutes (such as coral and hydroxyapatite ceramics), polysaccharides and microspheres and liposomes (Gittens & Uludag 2001; Malafaya et al 2002a, b; and this review). Given the complex interplay between cells, their environment, and growth factors, within the immediate in-vivo environment of a whole organism, an understanding of the limitations, issues and potential of each delivery system will be critical in harnessing the regeneration capacity of bone. This review will address the current advances, limitations and challenges in growth factor delivery for skeletal regeneration and the potential of innovative delivery matrices and enabling technologies for healthcare advancement.

Clinical target

The clinical need for efficacious delivery of growth factors and osteotropic agents, in co-ordination with cell therapies, is evidenced by the fact that tissue loss, as a result of injury or disease, leads to a reduced quality of life for many and is associated with significant social and economic costs. Each year in the UK there are some 150 000 fractures (wrist, vertebral and hip) due to osteoporosis. Hip fracture, in particular, is associated with high morbidity and mortality with fewer than half the patients returning home after surgery. Jordan &

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Cooper (2002) have estimated the healthcare costs in treating patients with these bone fractures, in the UK alone, to be £1.7 billion. The paucity of techniques in reconstructive surgery and trauma emphasise the need for effective delivery strategies to drive the osteogenic process. However, the high cost of many of the recombinant osteotropic agents, combined with their remarkably short half-lives within the in-vivo arena, necessitates the development of efficacious and cost-effective growth factor delivery strategies.

Bone growth factors for skeletal regeneration

Bone is characterised by an extraordinary potential for growth, regeneration and remodelling throughout life. This is largely due to the directed differentiation of mesenchymal cells into osteogenic cells, a process subject to exquisite regulation and complex interplay by a variety of hormones and differentiation factors present within the bone matrix (Bianco & Robey 2001; Bianco et al 2001). The following osteogenic growth factors (by no means an exhaustive list) have been identified as clinically important players in bone regeneration.

Transforming growth factor beta

Transforming growth factor beta (TGF-β) is a potent multifunctional 25 kD polypeptide synthesised in an inactive form (latent TGF-β) bound to latent TGF-β binding protein (for an extensive review see Bonewald 2003). Four distinct isoforms (TGF-β1, -β2, -β3 and -β5) exist and the polypeptide has been shown to stimulate osteoblast proliferation and matrix synthesis (including mineralization). TGF-β, sequestered within the bone matrix, is a significant player in the generation of committed osteoblast cells from determined osteogenic precursor cells (Pfeilschifter et al 1987, 1990a, 1993; reviewed in Bonewald 2003). It has been shown to bind to distinct serine-threonine kinase receptors resulting in activation of Smad 2 and 3, which subsequently bind to Smad 4 to modulate transcription. The ability of osteoclasts to activate latent TGF-β (Oreffo et al 1989; Pfeilschifter et al 1990b; Dallas et al 2002) during bone resorption to release active TGF-β, to stimulate osteoblast function and bone formation, suggests this polypeptide could be pivotal in the coupling of bone formation and bone resorption during the process of bone remodelling. Administration, in-vivo, has been shown to result in new bone formation when placed close to bone in models such as the calvarial bone formation (Noda & Camilliere 1989; Marcelli et al 1990) or skull defect models (Beck et al 1991). Furthermore, systemic administration of TGF-β2 will stimulate bone formation in the non-load-bearing rat and reverses the bone loss observed in osteoporotic mice. However, TGF-β cannot initiate the bone formation cascade in extraskeletal sites like the bone morphogenetic proteins (Reddi 1992).

Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs), originally identified as proteins present in demineralised bone matrix that could induce chondrogenesis and osteogenesis when implanted in extraskeletal sites, are key osteoinductive factors pivotal in the recruitment, commitment and differentiation of osteoprogenitors (Wozney et al 1988; Hollinger et al 1998; Wozney & Rosen 1998). Over 30 members have been identified and, with the exception of BMP-1, the BMPs are structurally related to the TGF-β superfamily, which includes TGF-βs, activins and inhibins, as well as Mullerian inhibiting substance. The BMPs, like the TGF-β proteins, are found in the bone matrix and are synthesised by skeletal and extraskeletal tissues as larger precursor molecules, which are processed to 30 kD dimers before their secretion from the cell. The BMPs are recognised as the primordial signalling molecules initiating bone cell differentiation from inducible mesenchymal stem cells, enhancing osteoblast differentiation and finally terminal osteoblastic differentiation. Clinical interest has centred particularly on BMP-2 and -4 in the potential treatment of fracture repair, large segmental bone defects, spinal fusion and in the fixation of prosthetic implants (Wozney et al 1988; Yasko et al 1992; Hollinger et al 1998; Hollinger & Winn 1999). BMP-2 has been shown to induce bone formation in monkey muscle, rat segmental non-union defects, and human femoral non-unions (Boden 1999; Ludwig & Boden 1999; Martin et al 1999). Boden et al (1995), in a small clinical trial, have already shown clinical efficacy for BMP-2 in spinal fusions (interbody fusion cages) comparable with autograft after a period of 24 months. Recently, the BESTT study (BMP-2 Evaluation in Surgery for Tibial Trauma), a prospective, controlled and randomized study of 450 patients, indicated that recombinant human BMP-2 was safe, well tolerated and demonstrated earlier functional recovery with reduced infection rates (Govender et al 2002). Interestingly, as recently reviewed by Nakashima & Reddi (2003), BMPs alone appear to be sufficient for the regeneration of dental tissues in adults. A number of studies from Ripamonti's laboratory, using a baboon model, indicate that BMP-7 can be used to regenerate bone in molars of these animals and that periodontitis induced in the molars can be treated with BMP-7/type I collagen composites (Ripamonti et al 2001a, b). Given the approval by the FDA of rhBMPs for accelerating bone fusion in slow-healing fractures, the stage is set for the application of BMPs in endodontics and periodontal surgery. The applications of BMPs in orthopaedics continue to generate significant interest with potential in fracture repair, large segmental bone defect repair and spinal fusion, as well as in the fixation of prosthetic implants, if delivery and maintenance of efficacy can be achieved.

Osteoblast-stimulating factor-1/pleiotrophin

Osteoblast-stimulating factor-1 is a secreted lysine- and cysteine-rich peptide, also referred to as heparin-binding growth associated molecule or more commonly known as pleiotrophin (PTN) (Deuel et al 2002). PTN is highly conserved across species with more than 90% homology among chicken, rat, bovine and human sequences and is sequestered in appreciable quantities (3.5 mg kg^{-1} wet weight) within the bone matrix. PTN was shown by Imai et al (1998) to be a matrix-bound chemotactic signalling molecule for osteoblastic cells while Yang et al (2003a) showed PTN has the ability to promote the adhesion, migration and differentiation of human osteoprogenitor cells. Studies from Tare et al (2002a, b) indicate that PTN

is an accessory signalling molecule, involved in bone formation, capable of modulating osteoprogenitor response to BMPs dependent on time of addition. Previous data has indicated that PTN over-expression in transgenic mice led to an increase in bone mineral content, enhanced epiphyseal function and was protective of transgenic mice subjected to oestrogen withdrawal as a consequence of ovariectomy (Masuda et al 1997). In addition, the demonstration of new bone formation by human osteoprogenitors on PTN-adsorbed PLGA scaffolds in rodent models indicates the potential of this growth factor in bone tissue engineering (Tare et al 2002a, b).

Local growth factors

In addition to TGF-β, BMPs and PTN, a variety of osteogenic growth factors, including insulin-like growth factors (IGFs), fibroblast growth factors (FGFs) and platelet-derived growth factor (PDGF) are also sequestered within the bone matrix and are implicated in bone formation. The insulin-like growth factors (IGFs), IGF-I and IGF-II, are 7.6 kD polypeptides synthesised by skeletal and non-skeletal tissues that act independently to stimulate bone collagen synthesis in bone cells, as well as osteogenesis and chondrogenesis (Linkhart et al 1996; Mohan & Baylink 1996). The activity of both IGF-I and IGF-II are regulated by a variety of factors, particularly the high affinity IGF-binding proteins (designated IGFBP-1 to -6: Hwa et al 1999). A number of in-vitro and in-vivo studies on hypophysectomised or ovariectomised rats have shown IGF-I will stimulate bone formation, while IGF-I null mice have decreased bone formation and transgenic mice over-expressing IGF-I display increased bone formation (Zhao et al 2000; Bikle et al 2002). FGF-1 and FGF-2 are potent mitogens for cells of the osteoblast lineage stimulating proliferation and augmenting the effects of dexamethasone (Pitaru et al 1993). Observations of impaired bone formation and decreased osteoblast number in FGF null mice, as well as stimulation of fracture repair by rhFGF in normal and diabetic mice, indicate that FGF-2 has an important role in bone repair (Montero et al 2000) as well as its recognised angiogenic properties. PDGF, a 30 kD polypeptide, like the FGFs, is a potent mitogen for bone cells enhancing proliferation, alkaline phosphatase activity and chemotaxis, as well as collagen activity (Centrella et al 1989; Canalis et al 1992). PDGF is composed of two polypeptide chains, derived products of distinct but related genes (PDGF-A and PDGF-B). Thus PDGF can exist as either PDGF-AA or PDGF-BB homodimers or as a heterodimer (PGDF-AB). PDGF is a potent mitogen implicated in wound healing and may be efficacious if administered for the topical treatment of bone disorders. Thus, local application of recombinant PDGF-BB was found to accelerate the healing of tibial osteotomy in rabbits and, when applied with IGF-I, was shown to enhance healing and new periodontal bone formation in the mandible after periodontal surgery in beagle dogs (Lynch et al 1991; Nash et al 1994). Phase I/II clinical trials of PDGF in combination with IGF-I, in patients with periodontal disease, showed enhanced periodontal bone formation (Howell et al 1997). However, the effectiveness of PDGF alone in fracture repair remains unclear.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a 34–46 kD homodimeric glycoprotein and is an essential mediator of angiogenesis (Fong et al 1995; Ferrara 2000; Ferrara et al 2003). VEGF is distantly related to PDGF and four additional homologous VEGF family members, including placental growth factor (PLGF), VEGF-B, VEGF-C and VEGF-D, have been identified. Three homologous, high affinity, transmembrane receptors to different subsets of the known VEGFs have also been identified: VEGFR1 (also known as Flt-1), VEGFR2 (also known as Flk-1 and KDR in mouse and man, respectively) and Flt-4 (VEGF-3) (Ferrara et al 2003). Mouse embryos lacking VEGFR2 or VEGFR1 die in-utero between days 8.5 and 9.5 (Ferrara et al 2003). Flt-1 appears to be required for endothelial assembly into functional blood vessels, while Flk/KDR plays a crucial role in vasculogenesis through modulation of angioblast formation. The first step in epiphyseal bone formation is proliferation of blood vessels in cartilage (the chondroepiphysis). This process of angiogenesis is essential for the recruitment of cell types involved in cartilage resorption and bone formation and almost certainly provides the necessary signal mechanisms for bone growth (Roach et al 1998). At the growth plate, angiogenesis occurs at the zone of hypertrophic chondrocytes, cells known to express VEGF (Gerber et al 1999) and ideally placed to induce vascular invasion. Seminal studies from Gerber et al (1999) demonstrated that administration of a soluble chimeric receptor (Flt-(1–3)-IgG) to VEGF, to 24-day-old mice, suppressed capillary invasion, impaired cartilage remodelling, and impaired bone formation. VEGF has a direct effect on osteoblast differentiation and function (Midy & Plouet 1994; Wang et al 1997), thus VEGF plays a pivotal role in the coordination of chondrocyte and osteoblast function, angiogenesis in the epiphyseal growth plate and bone formation. Therefore, VEGF could prove central in new bone formation strategies in combination with inductive bone agents.

Issues in growth factor delivery

Pivotal to providing replacement tissues as an alternative therapy for bone regeneration will be the specific delivery of growth factors, such as those detailed above, to enhance bone formation either in-vivo or in-vitro before implantation (Gittens & Uludag 2001; Malafaya et al 2002a, b). Therefore, to design and develop a controlled delivery system for tissue engineering purposes, a number of key issues must be addressed: an appropriate carrier system is required; incorporation of the protein into the carrier system without loss of protein activity is essential; the optimum growth factor cocktail and dosage must be known; and the release profile from the carrier has to stimulate and sustain tissue regeneration (Whitaker et al 2001).

Carrier systems

The optimal tissue engineering scaffold would include a porous support mechanism for tissue growth with a controlled delivery system incorporated into that porous network. When incorporating a protein within a scaffold, the

protein can either be immobilised on the scaffold itself or encapsulated within a delivery system, which is incorporated within the scaffold matrix (or a combination of the two approaches). Current drug delivery systems designed to administer drugs to the patient provide a strong basis for developing mechanisms for encapsulating growth factors for tissue engineering (Saltzman & Olbricht 2002). Incorporation into polymeric devices can often stabilise the protein, maintaining it in its biologically active state before and during the period of release. Use of excipients or co-dispersants can further stabilise the protein in the matrix (Anseth et al 2002). However, protein degradation during the encapsulating or scaffold fabrication route can be an issue as many of these methods involve high temperatures (such as the ceramics) or organic solvents (polymeric devices) (Fu et al 1999). A number of approaches have been used to circumvent these problems, including soaking the scaffold in a solution of growth factor after processing (Itoh et al 2001), use of hydrogel delivery systems where growth factor incorporation can be achieved at low temperatures (Ruel-Gariepy et al 2000; Molina et al 2001; Chen & Mooney 2003) and supercritical carbon dioxide processing of the polyesters (Mooney et al 1996; Howdle et al 2001); the latter has been shown to generate osteoconductive polymer scaffolds used successfully to generate new bone formation (Yang et al 2003b, c).

Functionality

Scaffolds loaded with growth factors often exhibit an initial burst of growth factor release followed by maintained delivery, which not only delivers the growth factor to the desired site of regeneration but helps to maintain biological activity. Typically, however, the growth factor release profile is poor in-vivo with observed short half-lives (Meinel et al 2001; Singh et al 2001). Large proteins often diffuse slowly through tissues, and so generating a scaffold with growth factor incorporated throughout overcomes this issue. However, scaffolds with large pore sizes may not be able to retain sufficient quantities of protein and so a combination of scaffold and drug-loaded microspheres has been demonstrated to address this problem (Bordem et al 2001). This approach is especially useful for the delivery of low-molecular-weight or water-soluble drugs (Malafaya et al 2002b). Using a scaffold as a drug carrier is also essential for delivering growth factors that act locally, such as the BMPs (Li & Wozney 2001). Central in such a strategy is the ability of the growth factor to target the desired cell population and diffusion into non-target tissues is avoided.

Composition of factors and release

A further issue in growth factor delivery is determining the growth factor cocktail required for optimal bone regeneration. Growth factors must also be delivered within the therapeutic range to be effective (Jepsson & Aspenberg 1996). Most growth factors are incorporated within a delivery system in an individual fashion but with innervated and vascularised tissues such as bone, further growth factors aimed at angiogenesis and nerve regeneration will be essential. Indeed, encouraging a blood supply through any tissue-engineered scaffold is essential for tissue survival as it provides a route for growth factor entry once the scaffold has degenerated (Kirkpatrick et al 2003). This is a complex issue, however, as all growth factors can influence a variety of cell and tissue responses and it will be essential to ensure that the right cocktail is administered to generate the desired tissues in the right location. Relatively little has been reported on the release profile of growth factors and the subsequent influence on tissue regeneration and there is a need for further in-vitro and in-vivo correlation studies. Further work also needs to address the efficacy and safety of the various growth factors involved in bone regeneration.

Delivery matrices

The efficacy of a growth factor delivery matrix can be characterized by: the newly formed bone within the implantation site or within the scaffold; significantly reduced dose for efficacious bone induction; improved reproducibility of the bone regeneration cascade in the presence of osteoinductive growth factors; minimal bone induction at undesired sites; and no significant acute or chronic inflammation. As discussed, the delivery system can be incorporated into a scaffold device and the combination then functions as the substrate for tissue regeneration. Alternatively, the delivery system can serve as a scaffold by itself (Figure 1). Up to now, a variety of biomaterials and processing techniques have been used for the fabrication of growth factor and carrier composites, as summarised in Table 1 for BMP-2 and TGF-β delivery.

Synthetic polymeric matrices

The proven biocompatibility, known degradation profiles and tuneable mechanical properties make synthetic polymeric materials favoured matrices as sustained and localized delivery vehicles for growth factors in bone tissue engineering applications. Commonly used synthetic polymer carriers for growth factor delivery include the poly(alpha-hydroxy acids) (Miyamoto et al 1992, 1993; Hollinger & Leong 1996), PEG-based polymers made by ring-opening polymerization (Saito et al 1999, 2001a, 2003; Burdick et al 2002), Poloxamer 407 (Clokie & Urist 2000), lactic-acid-based networks (Burdick et al 2003), polyanhydrides (Lucas et al 1990), thermoreversible protein-conjugating polymers based on *N*-isopropylacrylamide (NiPAM), ethyl methacrylate (EMA) and *N*-acryloxysuccinimide (NASI) (Gao & Uludag 2001; Gao et al 2002) and polyphosphates, such as poly(bisphenol A-phenylphosphate) (Reiner & Kohn 1997). The manufacture of porous polymeric structures from these materials, for tissue engineering, can involve a number of different technologies. These include phase inversion processes (Hinrichs et al 1992), freeze-drying (Hou et al 2003), particulate leaching (Thomson et al 1995) and rapid prototyping techniques of polymer melts and powders (Giordano et al 1996; Hutmacher et al 2001; Xiong et al 2001), or combinations of the above. Several mechanisms are involved in the release kinetics of growth factor from the polymeric matrix, including diffusion, bulk polymer degradation, ion complexation, interactions between the protein and the polymer and

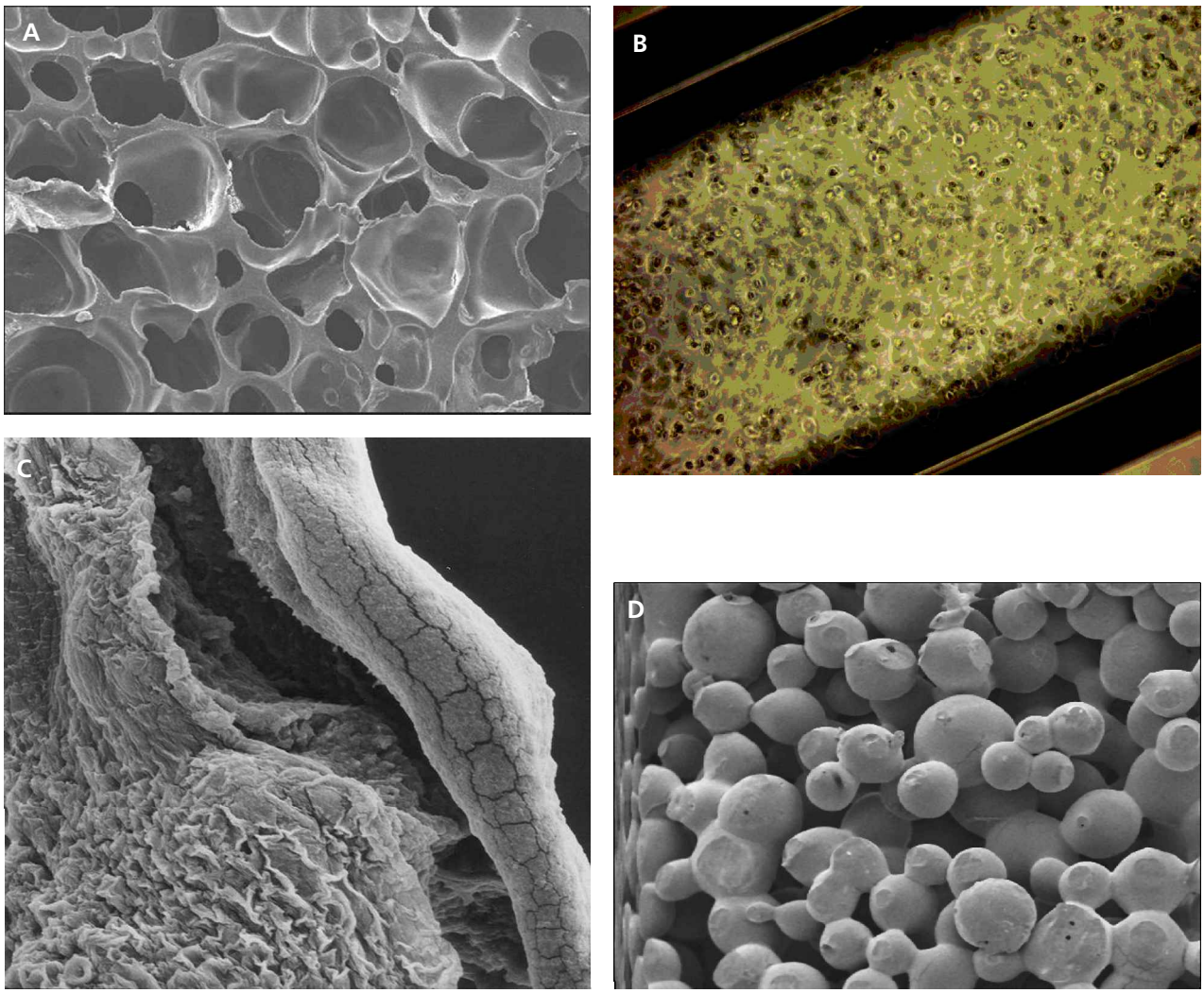

Figure 1 Tissue engineering scaffolds used for the delivery of bone growth factors. A. PLA scaffold produced using supercritical fluid technology. B. Collagen hydrogel. C. Alginate/chitosan scaffold. D. PLA/PEG microparticle based scaffold. Images by: Marta Silva (A), Clean Technology Group, School of Chemistry, University of Nottingham (reproduced with the permission of Professor Steven Howdle); Tri Suciati (D) and Katrina Teare (B), Tissue Engineering Group, School of Pharmacy, University of Nottingham (reproduced with the permission of Professor Kevin Shakesheff); David Green (C), University Orthopaedics, University of Southampton (reproduced with the permission of Dr David Green and Dr Richard OC Oreffo).

media/implant site components (such as enzymes). Approaches to address the degradation characteristics of the excipient combined with retaining biological activity of the growth factor have included the development of poly-D,L-lactic acid-*p*-dioxanone-polyethylene glycol block copolymer scaffolds (PLA-DX-PEG) (Saito et al 2001b) and PEG-based synthetic hydrogels that show promising degradation characteristics for delivery of rhBMP-2 (Lutolf et al 2003). Further considerations include the type of growth factor incorporated and the physicochemical properties of carrier used, including pore size and its distribution, porosity and charge density on the surface. In addition, the bone cell response is tightly related to the sequence and timing of growth factor delivery (Kim & Valentini 1997). The processing method used to generate a scaffold while incorporating growth factors also plays an important role in the efficacy of the delivery system. The carrier system should preferably be prepared under mild conditions to maintain high bioactivity of the growth factors incorporated and to avoid aggregation of the protein. For example, supercritical fluid technology to foam poly-(D,L-lactic acid) and growth factors in a one-step process (Figure 1A) (Howdle et al 2001; Whitaker et al 2001) and photoencapsulation of growth factors within in-situ forming degradable hydrogels based on PEG (Burdick et al 2002, 2003) have been used for the preparation of growth factor delivery matrices at low temperatures and use no solvents, which results in a high level of bioactivity of the released proteins. Moreover, injectable growth factor carriers

Table 1 Delivery matrices for BMP-2 and TGF-β in bone tissue engineering.

Carrier	Tissue regenerated	Reference
BMP-2		
Collagen	Ectopic bone formation	Takaoka et al (1991)
IBGS	Lumbar spinal fusion	Boden et al (1995)
Collagen, IBGS and polymers (& their composites)	Dorsum of the lumbar spine	Clokie & Urist (2000)
PDLLA and collagen sponges	Sheep cervical spine	Kandziora et al (2002), Schmidmaier et al (2002)
Injectable PLA-PEG	Mouse orthotopic bone	Saito et al (2003)
PLA-DX-PEG	Orthotopic bone	Saito et al 2001b
PEG based hydrogels	Rat crania	Lutolf et al (2003)
PLGA	Segmental rat femur defects	Lee et al (1994), Kirker-Head et al (1998)
PLGA	Ectopic bone formation	Whang et al (1998)
Type I collagen, PDLLA & IBGS	Rat facial planes	Winn et al (1999)
HA/collagen composite	Radius	Itoh et al (2001)
Bone ceramics and collagen	Lumbar spinal fusion	Minamide et al (2001)
Hyaluronic acid scaffold	Spinal fusion	Kim & Valentini (2002)
Type 1 collagen	Rabbit unilateral critical-sized defects	Hollinger et al (1998), Minamide et al (2001)
Absorbable collagen sponge	Maxillary floor sinus augmentation	Boyne et al (1997)
PDLLA scaffold	Critical-sized defects in radii of rabbits	Wheeler et al (1998)
PLGA microspheres	Rat calvarial defects	Woo et al (2001)
Magnetic rhBMP-2 liposomes	Rat segmental bone defects	Matsuo et al (2003)
Gelatin	Ectopic bone formation	Yamamoto et al (1998, 2003)
PLA encapsulated BMP-2 ($scCO_2$)	Ectopic bone formation (sub-cutaneous)	Yang et al (2003b)
HBM (adenovirally transduced) on PLGA/PLA scaffolds	Bone formation in diffusion chamber	Howard et al (2002)
In-situ forming PDLLA-b-PEG-b-PDLLA networks	Rat cranial defect	Burdick et al (2002)
Hyaluronic acid	Ectopic bone formation	Bulpitt & Aeschlimann (1999)
Rat DMB	Segmental bone defects in rats	Yasko et al (1992)
TGF-β		
EVA copolymer	Foetal-rat bone	Kim & Valentini (1997)
PLLA/alginate membrane	Bone defects	Milella et al (2001)
PLGA & DBM	Bone healing	Gombotz et al (1993)
Gelatin	Rabbit calvarial defect	Tabata et al (1998), Yamamoto et al (2000)
Collagen sponge	Skull defects	Ueda et al (2002)
PPF porous scaffolds	Rabbit cranial defect	Vehof et al (2002)
PLGA/PEG microparticles	Bone defects	Lu et al (2000)
Coral IBGS	Rat ectopic	Demers et al (2002)

BMP, bone morphogenetic protein; DBM, demineralised bone matrix; EVA, ethylene-vinyl acetate copolymer; HA, hydroxyapatite; HBM, human bone marrow; IBGS, inorganic bone graft substitute; PDLLA, poly(D,L-lactide); PEG, poly(ethylene glycol); PLA-DX-PEG, Poly-D,L-lactic acid-*p*-dioxanone-polyethylene glycol block copolymer; PLGA, poly(DL-lactic-co-glycolic acid); PLLA, Poly(L-lactic acid); PPF, poly(propylene fumarate); TGF, transforming growth factor.

can be prepared easily, by polymerising the hydrogel in the presence of the growth factor (Han & Hubbell 1996) or by solvent evaporation techniques (Saito et al 2001a), and applied to defects in a minimally invasive manner (Matsuo et al 2003). Where solvents are used, the growth factor can be protected from degradation during processing by the addition of other proteins, such as albumin, to act as a carrier and therefore stabilise the growth factor (Kim & Valentini 1997). Clearly the ability to create delivery systems that release growth factors in response to mechanical signals (Lee et al 2000) and deliver multiple growth factors with distinct kinetics (Richardson et al 2001) remains an exciting area for development in skeletal regeneration.

Collagen

Collagen is among the most commonly used natural materials for controlled delivery of bone growth factors (Boyne 1996, 2001; Boyne et al 1997; Hollinger et al 1998). It is often cross-linked to form a sponge to improve mechanical stability and resistance against degradation in-vivo

(Jorge-Herrero et al 1999) or used as a hydrogel (Figure 1B). Cross-linking can be achieved using chemicals such as glutaraldehyde, although this can be cytotoxic to cells and so other chemicals, such as diphenylphosphoryl azide (DPPA) have been assessed (Marinucci et al 2003). The scaffolds are then usually sterilised using ethylene oxide before adsorption of the growth factor by soaking the scaffold in the protein solution (Uludag et al 2001). The cross-linking and the sterilization processes play an important role in the chemical and physiochemical properties of the resulting scaffold and the incorporation of growth factors within it (Friess et al 1999a; Ueda et al 2002). Other processing parameters, such as growth factor concentration, product pH or anion concentration, and salt concentration in the expressed solution, also need to be considered to achieve maximal and consistent binding and to avoid precipitation (Friess et al 1999b; Uludag et al 2001). Using rhBMP as an example, the release pharmacokinetics from a collagen sponge are predominantly dependent on the characteristics of the growth factor itself (Uludag et al 1999a, b). Initial retention of rhBMP within an implant has also been shown to be dependent on the isoelectric point (pI) — the higher the pI, the higher the amount retained in the implant site, which in turns leads to more bone regeneration (Uludag et al 2000, 2001).

Inorganic bone graft substitutes

Perhaps the best examples of growth factor delivery matrices are the inorganic bone graft substitutes, which have been extensively evaluated for bone regeneration due to their physicochemical similarities to bone mineral and their proven osteoconductivity (Oonishi 1991). These include calcium phosphate cements (CPCs) (Blom et al 2002), hydroxyapatite (HA) and carbonated apatite (CA) (Arm et al 1996; Midy et al 1998), tricalcium phosphate (TCP) (Lind et al 2001) and silica carriers (Santos et al 1999). Due to the high sintering temperatures normally associated with the manufacture of scaffolds from these materials, or that they are sourced from ready made natural materials (such as coral or bovine bone), growth factor incorporation is mainly achieved by adsorption post fabrication. Subsequent release is largely diffusion-controlled and is typically a two-phase pattern of an initial burst release, followed by a specific release, which is dependent upon the physical and chemical interaction between the material and the growth factor and the adsorption conditions (Demers et al 2002; Ziegler et al 2002). A number of in-vivo studies indicate the efficacy of these scaffolds, including the use of sintered bovine bone (true bone ceramics) coated with type I collagen to deliver rhBMP to facilitate lumbar spinal fusion in a rabbit model (Minamide et al 2001) and the incorporation of rhBMP with alpha-BSM(R) (a biomimetic endothermically setting apatitic calcium phosphate) in promoting bone regeneration and accelerating restoration of the differentiated phenotype in an osteotomy model (Lee et al 1999).

Polysaccharide-based scaffolds

To address the limitations associated with commonly used growth factor delivery systems, such as the bone graft substitutes, including early burst release and protein degradation in biological environments, incorporation of growth factors into slow-release polysaccharide-based scaffolds have been reported. Such polysaccharides include alginate, hyaluronic acid, arabinogalactan, dextran and amylose, which may be synthesized into sponges by cross linking with chitosan, gelatin or bovine serum albumin (Ehrenfreund-Kleinman et al 2003) or extruded into fibres. Growth factors can then be entrapped within the scaffold by adsorption or by using emulsion techniques, as recently demonstrated with hyaluronic acid-based and chitosan-based scaffolds for sustained growth factor delivery (Lisignoli et al 2001, 2002; Marinucci et al 2001; Kim & Valentini 2002). The maintenance of high levels of bioactivity within the implantation site in these studies demonstrated enhanced progenitor cell differentiation and subsequent bone regeneration. Site-specific delivery of BMP has also been achieved by coupling a novel BMP-2-derived oligopeptide (NSVNSKIPKACCVPTELSAI) to alginate, which was shown to promote ectopic bone formation following implantation into the calf muscle of rats (Suzuki et al 2000). Recent studies from Green et al (2003) indicate the potential to combine alginate and chitosan to generate mineralised polysaccharide capsules that can be exploited to encapsulate a range of cell types, including promyoblasts, chondrocytes, adipocytes, immunoselected mesenchymal stem cells and the osteogenic factor, rhBMP-2, without loss of function in-vitro and in-vivo (Figure 1C). Furthermore, by controlling the extent of mineralization within the alginate/chitosan shell membrane, degradation of the shell wall and release of cells or osteogenic growth factor (rhBMP-2) into the surrounding medium could be regulated. Porous chondroitin-4-sulfate (CS) chitosan sponges have been shown to deliver sustained release of PDGF-BB and BMP to promote bone regeneration (Muzzarelli et al 1997; Park et al 2000a). By changing the CS composition or the initial loading content of the growth factor within the CS sponge, growth factor release could be controlled, improving osteoblast proliferation, osteoconductivity and new bone formation (Park et al 2000b; Lee et al 2002).

Microspheres and liposomes

Microspheres and liposomes are frequently used in drug and protein delivery systems. The growth factors released from these matrices maintain a high level of biological activity and the local retention of growth factor at the preferred site is enhanced for an extended period of time (Ditizio et al 2000). The well established double-emulsion–solvent-encapsulation technique has been used to encapsulate rhBMP-2 within PLGA microspheres and the subsequent controlled release of this growth factor has led to more effective and complete bone healing in animal models (Oldham et al 2000; Woo et al 2001; Weber et al 2002). Encapsulation of proteins using this method involves dissolving the growth factor in the water phase and the polymer, usually a polyester, is dissolved in the solvent phase before the two solutions are mixed at appropriate ratios. A second emulsion is then formed by dispersion in an aqueous phase using homogenisation or sonication. This emulsion is then stirred to evaporate the solvent, forming microspheres that can be isolated by centrifugation or filtration and can then be sintered to produce a scaffold

(Figure 1D) (Bordem et al 2001). Further studies have incorporated PEG into the microparticles to fine tune growth factor release (Lu et al 2000) and included the addition of stabilisers to reduce surface aggregation and therefore improve the retention of the growth factor within the microparticles (Fu et al 1999; Bezemer et al 2000). Growth factors can also be incorporated within liposomes at the time of manufacture using the reverse-phase evaporation method followed by sonication. In two separate studies using magnetic liposomes or liposomes encapsulated within alginate to modulate growth factor release, enhanced retention of growth factors at the implantation site was observed (Matsuo et al 2003; Dhoot & Wheatley 2003).

Gene therapy as a method of growth factor delivery

The ability to transfer specific bone growth factor genes into multipotential mesenchymal stem cells, while still in its infancy, offers considerable therapeutic hope in a variety of musculoskeletal disorders. Gene delivery essentially involves either viral transduction or non-viral transfection. These methods have varying means to overcome the extracellular and intracellular barriers of delivery of DNA to the nucleus. The process by which foreign DNA is taken up by cells begins with adsorption of the DNA to the cell surface whether it is delivered by a viral vector or complexed with various transfection reagents. After nuclear translocation through nuclear pores takes place, the foreign DNA will either be integrated into the host genome or remain episomal. Transport across the nuclear membrane involves pathways mediated by various carrier proteins, is a signal-mediated process and is potentially the greatest barrier to exogenous DNA. Viral approaches to gene transfer have a history of efficacy. Both retroviral and adenoviral delivery systems have been developed and numerous examples of adenoviral delivery of BMP-2 to mesenchymal stem cells in-vitro and in-vivo exist, with formation of histologically and X-ray positive bone tissue. However, viral delivery carries concerns of immunogenicity and mutagenesis, which will limit its usefulness in-vivo.

Non-viral gene delivery strategies for tissue engineering are in the very earliest stages of development and include cationic polymers, lipid based polymers, biomaterials such as chitosan and physical methods such as electroporation (reviewed in Partridge & Oreffo 2004). All of these methods are less efficient than viral transduction and there are viability complications due to toxicity. However, gene delivery by a non-viral route is not immunogenic and there are few restrictions on the size of the gene of interest that can be delivered. Additional advantages to non-viral gene delivery include the ability to target specific tissues and low toxicity, although only a few bone tissue specific examples have been published (Partridge & Oreffo 2004). To date, there is little information on the potential issues of the immune response to bacterially produced plasmid DNA and the ability to non-virally transfect primary mesenchymal cell populations remains to be optimised. The next decade will see the development of simple, safe and reproducible strategies for gene delivery that should address the current pressing orthopaedic clinical imperatives.

Future Perspectives

To generate affordable, readily available scaffolds for specifiable non-immunogenic transplantable tissues or as significant cell or growth delivery matrices, approaches to create smart or functional scaffold-guided tissue composites will be paramount. A number of factors will need to be addressed for this to be achieved. A combination of growth factors released from the matrix in a phased and sequential manner will be required for optimal expression of osteoactivity (Raiche & Puleo 2004) and the modulation of stem/progenitor cell populations. It will therefore be essential to understand the inter-relationships between different growth factors and how they exhibit their synergetic effect on bone healing and regeneration (Winn et al 1998; Arnold et al 2003). The recognition of the potential to modulate cell adhesion, proliferation and differentiation through select cues from integrin signalling to chemotactic manipulation offers new approaches to generate scaffolds targeted at specific cell population recruitment and differentiation. Furthermore, understanding the influence of the native in-vivo environment on the implanted matrix, such as the potential exploitation of cell-mediated proteolytic degradation (using cell matrix metalloproteinases) in synthetic mimetic matrices containing osteogenic growth factors to regenerate bone, as elegantly described by Lutolf et al (2003) will be essential.

Summary

Successful in-situ tissue growth and regeneration is dependent, in large part, on the ability to deliver specific growth factors to cells within regenerating tissues and a requirement to promote angiogenesis and vascularisation. Thus, strategies in tissue engineering have emerged for the development of sophisticated growth factor delivery mechanisms to mimic the endogenous profiles of growth factor production during natural tissue morphogenesis or regeneration. The successful generation of 3-D biomimetic structures incorporating slow-release osteoinductive factors indicates the potential for de-novo bone formation and the potential for more complex tissue regeneration strategies that will incorporate temporal and sequential release strategies for the augmentation of tissue regeneration that will, undoubtedly, be critical in the coming years. The challenge facing material scientists, biochemical engineers, cell biologists and clinicians will be the development of multidisciplinary approaches that integrate cell, molecular, biochemical and clinical techniques for tissue engineering — the impacts in terms of advancement, healthcare costs and, more importantly, improved quality of life are immense.

References

Anseth, K. S., Metters, A. T., Bryant, S. J., Martens, P. J., Elisseeff, J. H., Bowman, C. N. (2002) *In situ* forming degradable networks and their application in tissue engineering and drug delivery. *J. Control. Release* **78**: 199–209

Arm, D. M., Tencer, A. F., Bain, S. D., Celino, D. (1996) Effect of controlled release of platelet derived growth factor from a

porous hydroxyapatite implant on bone ingrowth. *Biomaterials* **17**: 703–709

Arnold, U., Schweitzer, S., Lindenhayn, K., Perka, C. (2003) Optimization of bone engineering by means of growth factors in a three-dimensional matrix. *J. Biomed. Mater. Res.* **67A**: 260–269

Beck, L. S., Deguzman, L., Lee, W. P., Xu, Y., McFatridge, L. A., Gillett, N. A., Amento, E. P. (1991) TGF-beta 1 induces bone closure of skull defects. *J. Bone Miner. Res.* **6**: 1257–1265

Bezemer, J. M., Radersma, R., Grijpma, D. W., Dijkstra, P. J., van Blitterswijk, C. A., Feijen, J. (2000) Microspheres for protein delivery prepared from amphiphilic multiblock copolymers. 1. Influence of preparation techniques on particle characteristics and protein delivery. *J. Control. Release* **67**: 233–248

Bianco, P., Robey, P. G. (2001) Stem cells in tissue engineering. *Nature* **414**: 118–121

Bianco, P., Riminucci, M., Gronthos, S., Robey, P. G. (2001) Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells* **19**: 180–192

Bikle, D. D., Sakata, T., Leary, C., Elalieh, H., Ginzinger, D., Rosen, C. J., Beamer, W., Majumdar, S., Halloran, B. P. (2002) Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. *J. Bone Miner. Res.* **17**: 1570–1578

Blom, E. J., Klein-Nulend, J., Wolke, J. G. C., Kurashina, K., van Waas, M. A. J., Burger, E. H. (2002) Transforming growth factor-beta 1 incorporation in an alpha-tricalcium phosphate/dicalcium phosphate dihydrate/tetracalcium phosphate monoxide cement: release characteristics and physicochemical properties. *Biomaterials* **23**: 1261–1268

Boden, S. D. (1999) Bioactive factors for bone tissue engineering. *Clin. Orthop.* **367**: S84–S94

Boden, S. D., Schimandle, J. H., Hutton, W. C. (1995) The use of an osteoinductive growth factor for lumbar spinal fusion. 2. Study of dose, carrier, and species. *Spine* **20**: 2633–2644

Bonewald, L. Transforming growth factor beta. Bilezikian, J. P., Raisz, L. G., and Rodan, G. A. (2003) *Principles of bone biology*. Academic Press, San Diego, pp 903–918

Bordem, M. D., Khan, Y., Attawia, M., Laurencin, C. T. (2001) Tissue engineered microsphere-based matrices for bone repair: design, evaluation, and optimization. *Biomaterials* **23**: 551–559

Boyne, P. J. (1996) Animal studies of the application of rhBMP-2 in maxillofacial reconstruction. *Bone* **19**: S83–S92

Boyne, P. J. (2001) Application of bone morphogenetic proteins in the treatment of clinical oral and maxillofacial osseous defects. *J. Bone Joint Surg. Am.* **83A**: S146–S150

Boyne, P. J., Marx, R. E., Nevins, M., Triplett, G., Lazaro, E., Lilly, L. C., 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**: 10–25

Bulpitt, P., Aeschlimann, D. (1999) New strategy for chemical modification of hyaluronic acid: Preparation of functionalized derivatives and their use in the formation of novel biocompatible hydrogels. *J. Biomed. Mater. Res.* **47**: 152–169

Burdick, J. A., Mason, M. N., Hinman, A. D., Thorne, K., Anseth, K. S. (2002) Delivery of osteoinductive growth factors from degradable PEG hydrogels influences osteoblast differentiation and mineralization. *J. Controlled Release* **83**: 53–63

Burdick, J. A., Frankel, D., Dernell, W. S., Anseth, K. S. (2003) An initial investigation of photocurable three-dimensional lactic acid based scaffolds in a critical-sized cranial defect. *Biomaterials* **24**: 1613–1620

Canalis, E., Varghese, S., McCarthy, T. L., Centrella, M. (1992) Role of platelet derived growth factor in bone cell function. *Growth Regul.* **2**: 151–155

Centrella, M., McCarthy, T. L., Canalis, E. (1989) Platelet-derived growth factor enhances deoxyribonucleic acid and collagen synthesis in osteoblast-enriched cultures from fetal rat parietal bone. *Endocrinology* **125**: 13–19

Chen, R. R., Mooney, D. J. (2003) Polymeric growth factor delivery strategies for tissue engineering. *Pharm. Res.* **20**: 1103–1112

Clokie, C. M. L., Urist, M. R. (2000) Bone morphogenetic protein excipients: comparative observations on poloxamer. *Plast. Reconstr. Surg.* **105**: 628–637

Dallas, S. L., Rosser, J. L., Mundy, G. R., Bonewald, L. F. (2002) Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. *J. Biol. Chem.* **277**: 21352–21360

Demers, C. N., Tabrizian, M., Petit, A., Hamdy, R. C., Yahia, L. (2002) Effect of experimental parameters on the *in vitro* release kinetics of transforming growth factor beta 1 from coral particles. *J. Biomed. Mater. Res.* **59**: 403–410

Deuel, T. F., Zhang, N., Yeh, H. J., Silos-Santiago, I., Wang, Z. Y. (2002) Pleiotrophin: a cytokine with diverse functions and a novel signalling pathway. *Arch. Biochem. Biophys.* **397**: 162–171

Dhoot, N. O., Wheatley, M. A. (2003) Microencapsulated liposomes in controlled drug delivery: strategies to modulate drug release and eliminate the burst effect. *J. Pharm. Sci.* **92**: 679–689

Ditizio, V., Karlgard, C., Lilge, L., Khoury, A. E., Mittelman, M. W., DiCosmo, F. (2000) Localized drug delivery using crosslinked gelatin gels containing liposomes: factors influencing liposome stability and drug release. *J. Biomed. Mater. Res.* **51**: 96–106

Ehrenfreund-Kleinman, T., Domb, A. J., Golenser, J. (2003) Polysaccharide scaffolds prepared by crosslinking of polysaccharides with chitosan or proteins for cell growth. *J. Bioactive Compatible Polymers* **18**: 323–338

Ferrara, N. (2000) VEGF: an update on biological and therapeutic aspects. *Curr. Opin. Biotechnol.* **11**: 617–624

Ferrara, N., Gerber, H. P., LeCouter, J. (2003) The biology of VEGF and its receptors. *Nat. Med.* **9**: 669–676

Fong, G. H., Rossant, J., Gertsenstein, M., Breitman, M. L. (1995) Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* **376**: 66–70

Friess, W., Uludag, H., Foskett, S., Biron, R. (1999a) Bone regeneration with recombinant human bone morphogenetic protein-2 (rhBMP-2) using absorbable collagen sponges (ACS): Influence of processing on ACS characteristics and formulation. *Pharm. Dev. Technol.* **4**: 387–396

Friess, W., Uludag, H., Foskett, S., Biron, R., Sargeant, C. (1999b) Characterization of absorbable collagen sponges as rhBMP-2 carriers. *Int. J. Pharm.* **187**: 91–99

Fu, K., Griebenow, K., Hsieh, L., Klibanov, A. M., Langer, R. (1999) FTIR characterization of the secondary structure of proteins encapsulated within PLGA microspheres. *J. Control. Release* **58**: 357–366

Gao, T. J., Uludag, H. (2001) Effect of molecular weight of thermoreversible polymer on *in vivo* retention of rhBMP-2. *J. Biomed. Mater. Res.* **57**: 92–100

Gao, T. J., Kousinioris, N. A., Wozney, J. M., Winn, S., Uludag, H. (2002) Synthetic thermoreversible polymers are compatible with osteoinductive activity of recombinant human bone morphogenetic protein 2. *Tissue Eng.* **8**: 429–440

Gerber, H. P., Vu, T. H., Ryan, A. M., Kowalski, J., Werb, Z., Ferrara, N. (1999) VEGF couples hypertrophic cartilage remodelling, ossification and angiogenesis during endochondral bone formation. *Nat. Med.* **5**: 623–628

Giordano, R. A., Wu, B. M., Borland, S. W., Cima, L. G., Sachs, E. M., Cima, M. J. (1996) Mechanical properties of

dense polylactic acid structures fabricated by three dimensional printing. *J. Biomater. Sci. Polym. Ed.* **8**: 63–75

Gittens, S. A., Uludag, H. (2001) Growth factor delivery for bone tissue engineering. *J. Drug Target.* **9**: 407–429

Gombotz, W. R., Pankey, S. C., Bouchard, L. S., Ranchalis, J., Puolakkainen, P. (1993) Controlled-release of TGF-ß1 from a biodegradable matrix for bone regeneration. *J. Biomater. Sci. Polym. Ed.* **5**: 49–63

Govender, S., Csimma, C., Genant, H. K., Valentin-Opran, A., Amit, Y., Arbel, R., Aro, H., Atar, D., Bishay, M., Borner, M. G., 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, R. L., 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, P. M., Rondia, J., Rossouw, W. C., Daneel, P. J., Ruff, S., Ruter, A., Santavirta, S., Schildhauer, T. A., Gekle, C., Schnettler, R., Segal, D., Seiler, H., Snowdowne, R. B., 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-A**: 2123–2134

Green, D. W., Leveque, I., Walsh, D., Partridge, K. A., Mann, S., Oreffo, R. O. C. (2003) Innovative biomineralised polysaccharide templates for human bone cell and gene delivery. *J. Bone Miner. Res.* **18**: 1361

Han, D. K., Hubbell, J. A. (1996) Lactide-based poly(ethylene glycol) polymer networks for scaffolds in tissue engineering. *Macromolecules* **29**: 5233–5235

Hinrichs, W. L. J., Lommen, E., Wildevuur, C. R. H., Freijen, J. (1992) Fabrication and characterisation of an asymmetric polyurethane membrane for use as a wound dressing. *J. Appl. Biomater.* **3**: 287–303

Hollinger, J. O., Leong, K. (1996) Poly(alpha-hydroxy acids): Carriers for bone morphogenetic proteins. *Biomaterials* **17**: 187–194

Hollinger, J. O., Winn, S. R. (1999) Tissue engineering of bone in the craniofacial complex. *Ann. N. Y. Acad. Sci.* **875**: 379–385

Hollinger, J. O., Schmitt, J. M., Buck, D. C., Shannon, R., Joh, S. P., Zegzula, H. D., Wozney, J. (1998) Recombinant human bone morphogenetic protein-2 and collagen for bone regeneration. *J. Biomed. Mater. Res.* **43**: 356–364

Hou, Q., Grijpma, D. W., Feijen, J. (2003) Preparation of interconnected highly porous polymeric structures by a replication and freeze-drying process. *J. Biomed. Mater. Res.* **67B**: 732–740

Howard, D., Partridge, K., Yang, X. B., Clarke, N. M. P., Okubo, Y., Bessho, K., Howdle, S. M., Shakesheff, K. M., Oreffo, R. O. C. (2002) Immunoselection and adenoviral genetic modulation of human osteoprogenitors: *in vivo* bone formation on PLA scaffold. *Biochem. Biophys. Res. Commun.* **299**: 208–215

Howdle, S. M., Watson, M. S., Whitaker, M. J., Popov, M. C., Davies, M. C., Mandel, F. S., Wang, J. D., and Shakesheff, K. M. (2001) Supercritical fluid mixing: preparation of thermally sensitive polymer composites containing bioactive materials. *Chem. Commun.* **1**: 109–110

Howell, T. H., Fiorellini, J. P., Paquette, D. W., Offenbacher, S., Giannobile, W. V., Lynch, S. E. (1997) A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *J. Periodontol.* **68**: 1186–1193

Hutmacher, D. W., Schantz, T., Zein, I., Ng, K. W., Teoh, S. H. Tan, K. C. (2001) Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modelling. *J. Biomed. Mater. Res.* **55**: 203–216

Hwa, V., Oh, Y., Rosenfeld, R. G. (1999) The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocrinol. Rev.* **20**: 761–787

Imai, S., Kaksonen, M., Raulo, E., Kinnunen, T., Fages, C., Meng, X., Lakso, M., Rauvala, H. (1998) Osteoblast recruitment and bone formation enhanced by cell matrix-associated heparin-binding growth-associated molecule (HB-GAM). *J. Cell Biol.* **143**: 1113–1128

Itoh, S., Kikuchi, M., Takakuda, K., Koyama, Y., Matsumoto, H. N., Ichinose, S., Tanaka, J., Kawauchi, T., Shinomiya, K. (2001) The biocompatibility and osteoconductive activity of a novel hydroxyapatite/collagen composite biomaterial, and its function as a carrier of rhBMP-2. *J. Biomed. Mater. Res.* **54**: 445–453

Jepsson, C., Aspenberg, P. (1996) BMP-2 can inhibit bone healing – bone-chamber study in rabbits. *Acta Orthop. Scand.* **67**: 589–592

Jordan, K. M., Cooper, C. (2002) Epidemiology of osteoporosis. *Best Pract. Res. Clin. Rheumatol.* **16**: 795–806

Jorge-Herrero, E., Fernandez, P., Turnay, J., Olmo, N., Calero, P., Garcia, R., Freile, I., Castillo-Olivares, J. L. (1999) Influence of different chemical cross-linking treatments on the properties of bovine pericardium and collagen. *Biomaterials* **20**: 539–545

Kandziora, F., Scholz, M., Pflugmacher, R., Krummrey, G., Schollmeier, G., Schmidmaier, G., Schnake, K. J., Duda, G., Raschke, M., Haas, N. P. (2002) Experimental fusion of the sheep cervical spine. Part II: Effect of growth factors and carrier systems on interbody fusion. *Chirurg* **73**: 1025

Kim, H. D., Valentini, R. F. (1997) Human osteoblast response *in vitro* to platelet-derived growth factor and transforming growth factor-beta delivered from controlled-release polymer rods. *Biomaterials* **18**: 1175–1184

Kim, H. D., Valentini, R. F. (2002) Retention and activity of BMP-2 in hyaluronic acid-based scaffolds *in vitro*. *J. Biomed. Mater. Res.* **59**: 573–584

Kirker-Head, C. A., Gerhart, T. N., Armstrong, R., Schelling, S. H., Carmel, L. A. (1998) Healing bone using recombinant human bone morphogenetic protein 2 and copolymer. *Clin. Orthop.* **349**: 205–217

Kirkpatrick, C. J., Unger, R. E., Krump-Konvalinkova, V., Peters, K., Schmidt, H., Kamp, G. (2003) Experimental approaches to study vascularization in tissue engineering and biomaterial applications. *J. Mater. Sci. Mater. Med.* **14**: 677–681

Langer, R., Vacanti, J. P. (1993) Tissue engineering. *Science* **260**: 920–926

Lee, S. C., Shea, M., Battle, M. A., Kozitza, K., Ron, E., Turek, T., Schaub, R. G., Hayes, W. C. (1994) Healing of large segmental defects in rat femurs is aided by Rhbmp-2 in PLGA matrix. *J. Biomed. Mater. Res.* **28**: 1149–1156

Lee, D. D., Tofighi, A., Aiolova, M., Chakravarthy, P., Catalano, A., Majahad, A., Knaack, D. (1999) alpha-BSM (R): A biomimetic bone substitute and drug delivery vehicle. *Clin. Orthop.* **367**: S396–S405

Lee, J. Y., Nam, S. H., Im, S. Y., Park, Y. J., Lee, Y. M., Seol, Y. J., Chung, C. P., Lee, S. J. (2002) Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials. *J. Control. Release* **78**: 187–197

Lee, K. Y., Peters, M. C., Anderson, K. W., Mooney, D. J. (2000) Controlled growth factor release from synthetic extracellular matrices. *Nature* **408**: 998–1000

Li, R. H., Wozney, J. M. (2001) Delivering on the promise of bone morphogenetic proteins. *Trends Biotechnol.* **19**: 255–265

Lind, M., Overgaard, S., Glerup, H., Soballe, K., Bunger, C. (2001) Transforming growth factor-beta 1 adsorbed to trical-

ciumphosphate coated implants increases peri-implant bone remodeling. *Biomaterials* **22**: 189–193

Linkhart, T. A., Mohan, S., Baylink, D. J. (1996) Growth factors for bone growth and repair: IGF, TGF beta and BMP. *Bone* **19**: 1S–12S

Lisignoli, G., Zini, N., Remiddi, G., Piacentini, A., Puggioli, A., Trimarchi, C., Fini, M., Maraldi, N. M., Facchini, A. (2001) Basic fibroblast growth factor enhances *in vitro* mineralization of rat bone marrow stromal cells grown on non-woven hyaluronic acid based polymer scaffold. *Biomaterials* **22**: 2095–2105

Lisignoli, G., Fini, M., Giavaresi, G., Aldini, N. N., Toneguzzi, S., Facchini, A. (2002) Osteogenesis of large segmental radius defects enhanced by basic fibroblast growth factor activated bone marrow stromal cells grown on non-woven hyaluronic acid-based polymer scaffold. *Biomaterials* **23**: 1043–1051

Lu, L., Stamatas, G. N., Mikos, A. G. (2000) Controlled release of transforming growth factor beta 1 from biodegradable polymer microparticles. *J. Biomed. Mater. Res.* **50**: 440–451

Lucas, P. A., Laurencin, C., Syftestad, G. T., Domb, A., Goldberg, V. M., Caplan, A. I., Langer, R. (1990) Ectopic induction of cartilage and bone by water-soluble proteins from bovine bone using a polyanhydride delivery vehicle. *J. Biomed. Mater. Res.* **24**: 901–911

Ludwig, S. C., Boden, S. D. (1999) Osteoinductive bone graft substitutes for spinal fusion: a basic science summary. *Orthop. Clin. North. Am.* **30**: 635–645

Lutolf, M. R., Weber, F. E., Schmoekel, H. G., Schense, J. C., Kohler, T., Muller, R., Hubbell, J. A. (2003) Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. *Nat. Biotechnol.* **21**: 513–518

Lynch, S. E., de Castilla, G. R., Williams, R. C., Kiritsy, C. P., Howell, T. H., Reddy, M. S., Antoniades, H. N. (1991) The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. *J. Periodontol.* **62**: 458–467

Malafaya, P. B., Silva, G. A., Baran, E. T., Reis, R. L. (2002a) Drug delivery therapies I – general trends and its importance on bone tissue engineering applications. *Curr. Opin. Solid. St. M.* **6**: 283–295

Malafaya, P. B., Silva, G. A., Baran, E. T., Reis, R. L. (2002b) Drug delivery therapies II – Strategies for delivering bone regenerating factors. *Curr. Opin. Solid St. M.* **6**: 297–312

Marcelli, C., Yates, A. J., Mundy, G. R. (1990) In vivo effects of human recombinant transforming growth factor beta on bone turnover in normal mice. *J. Bone Miner. Res.* **5**: 1087–1096

Marinucci, L., Lilli, C., Baroni, T., Becchetti, E., Belcastro, S., Balducci, C., Locci, P. (2001) *In vitro* comparison of bioabsorbable and non-resorbable membranes in bone regeneration. *J. Periodontol.* **72**: 753–759

Marinucci, L., Lilli, C., Guerra, M., Belcastro, S., Becchetti, E., Stabellini, G., Calvi, E. M., Locci, P. (2003) Biocompatibility of collagen membranes crosslinked with glutaraldehyde or diphenylphosphoryl azide: an in vitro study. *J. Biomed. Mater. Res.* **67A**: 504–509

Martin, G. J., Boden, S. D., Marone, M. A., Moskovitz, P. A. (1999) Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman primate: important lessons learned regarding dose, carrier, and safety. *J. Spinal Disord.* **12**: 179–186

Masuda, H., Tsujimura, A., Yoshioka, M., Arai, Y., Kuboki, Y., Mukai, T., Nakamura, T., Tsuji, H., Nakagawa, M., Hashimoto-Gotoh, T. (1997) Bone mass loss due to oestrogen deficiency is compensated in transgenic mice overexpressing human osteoblast stimulating factor-1. *Biochem. Biophys. Res. Commun.* **238**: 528–533

Matsuo, T., Sugita, T., Kubo, T., Yasunaga, Y., Ochi, M., Murakami, T. (2003) Injectable magnetic liposomes as a novel carrier of recombinant human BMP-2 for bone formation in a rat bone-defect model. *J. Biomed. Mater. Res.* **66A**: 747–754

Meinel, L., Illi, O. E., Zapf, J., Malfanti, M., Merkle, H. P., Gander, B. (2001) Stabilizing insulin-like growth factor-I in poly(D,L-lactide-co-glycolide) microspheres. *J. Control. Release* **70**: 193–202

Midy, V., Plouet, J. (1994) Vasculotropin/vascular endothelial growth factor induces differentiation in cultured osteoblasts. *Biochem. Biophys. Res. Commun.* **199**: 380–386

Midy, V., Rey, C., Bres, E., Dard, M. (1998) Basic fibroblast growth factor adsorption and release properties of calcium phosphate. *J. Biomed. Mater. Res.* **41**: 405–411

Milella, E., Barra, G., Ramires, P. A., Leo, G., Aversa, P., Romito, A. (2001) Poly(L-lactide)acid/alginate composite membranes for guided tissue regeneration. *J. Biomed. Mater. Res.* **57**: 248–257

Minamide, A., Kawakami, M., Hashizume, H., Sakata, R., Tamaki, T. (2001) Evaluation of carriers of bone morphogenetic protein for spinal fusion. *Spine* **26**: 933–939

Miyamoto, S., Takaoka, K., Okada, T., Yoshikawa, H., Hashimoto, J., Suzuki, S., Ono, K. (1992) Evaluation of polylactic acid homopolymers as carriers for bone morphogenetic protein. *Clin. Orthop.* **278**: 274–285

Miyamoto, S., Takaoka, K., Okada, T., Yoshikawa, H., Hashimoto, J., Suzuki, S., Ono, K. (1993) Polylactic acid polyethyleneglycol block-copolymer – a new biodegradable synthetic carrier for bone morphogenetic protein. *Clin. Orthop.* **294**: 333–343

Mohan, S., Baylink, D. J. (1996) Insulin-like growth factor system components and the coupling of bone formation to resorption. *Horm. Res.* **45**: 59–62

Molina, I., Li, S. M., Martinez, M. B., Vert, M. (2001) Protein release from physically crosslinked hydrogels of the PLA/PEO/PLA triblock copolymer-type. *Biomaterials* **22**: 363–369

Montero, A., Okada, Y., Tomita, M., Ito, M., Tsurukami, H., Nakamura, T., Doetschman, T., Coffin, J. D., Hurley, M. M. (2000) Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation. *J. Clin. Invest.* **105**: 1085–1093

Mooney, D. J., Baldwin, D. F., Suh, N. P., Vacanti, L. P., Langer, R. (1996) Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents *Biomaterials* **17**: 1417–1422

Muzzarelli, R. A. A., Biagini, G., Belmonte, M. A., Talassi, O., Gandolfi, M. G., Solmi, R., Carraro, S., Giardino, R., Fini, M., NicoliAldini, N. (1997) Osteoinduction by chitosan-complexed BMP: Morpho-structural responses in an osteoporotic model. *J. Bioactive Compatible Polymers* **12**: 321–329

Nakashima, M., Reddi, A. H. (2003) The application of bone morphogenetic proteins to dental tissue engineering. *Nat. Biotechnol.* **21**: 1025–1032

Nash, T. J., Howlett, C. R., Martin, C., Steele, J., Johnson, K. A., Hicklin, D. J. (1994) Effect of platelet-derived growth factor on tibial osteotomies in rabbits. *Bone* **15**: 203–208

Noda, M., Camilliere, J. J. (1989) *In vivo* stimulation of bone formation by transforming growth factor-beta. *Endocrinology* **124**: 2991–2994

Oldham, J. B., Lu, L., Zhu, X., Porter, B. D., Hefferan, T. E., Larson, D. R., Currier, B. L., Mikos, A. G., Yaszemski, M. J. (2000) Biological activity of rhBMP-2 released from PLGA microspheres. *J. Biomech. Eng.* **122**: 289–292

Oonishi, H. (1991) Orthopaedic applications of hydroxyapatite. *Biomaterials* **12**: 171–178

Oreffo, R. O., Triffitt, J. T. (1999). Future potentials for using osteogenic stem cells and biomaterials in orthopedics. *Bone* **25**: 5S–9S

Oreffo, R. O., Mundy, G. R., Seyedin, S. M., Bonewald, L. F. (1989) Activation of the bone-derived latent TGF beta complex by isolated osteoclasts. *Biochem. Biophys. Res. Commun.* **158**: 817–823

Park, Y. J., Lee, Y. M., Lee, J. Y., Seol, Y. J., Chung, C. P., Lee, S. J. (2000a) Controlled release of platelet-derived growth factor-BB from chondroitin sulfate-chitosan sponge for guided bone regeneration. *J. Control. Release* **67**: 385–394

Park, Y. J., Lee, Y. M., Park, S. N., Sheen, S. Y., Chung, C. P., Lee, S. J. (2000b) Platelet derived growth factor releasing chitosan sponge for periodontal bone regeneration. *Biomaterials* **21**: 153–159

Partridge, K. A., Oreffo, R. O. C. (2004) Gene delivery in bone tissue engineering – progress and prospects using viral and non-viral strategies. *Tissue Eng.* **10**: In press

Pearson, R. G., Bhandari, R., Quirk, R. A., Shakesheff, K. M. (2002) Recent advances in tissue engineering: an invited review. *J. Long Term Eff. Med. Implants* **12**: 1–33

Peter, S. J., Miller, M. J., Yasko, A. W., Yaszemski, M. J., Mikos, A. G. (1998) Polymer concepts in tissue engineering. *J. Biomed. Mater. Res.* **43**: 422–427

Pfeilschifter, J., D'Souza, S. M., Mundy, G. R. (1987) Effects of transforming growth factor-beta on osteoblastic osteosarcoma cells. *Endocrinology* **121**: 212–218

Pfeilschifter, J., Wolf, O., Naumann, A., Minne, H. W., Mundy, G. R., Ziegler, R. (1990a) Chemotactic response of osteoblast like cells to transforming growth factor beta. *J. Bone Miner. Res.* **5**: 825–830

Pfeilschifter, J., Bonewald, L., Mundy, G. R. (1990b) Characterization of the latent transforming growth factor beta complex in bone. *J. Bone Miner. Res.* **5**: 49–58

Pfeilschifter, J., Diel, I., Pilz, U., Brunotte, K., Naumann, A., Ziegler, R. (1993) Mitogenic responsiveness of human bone cells *in vitro* to hormones and growth factors decreases with age. *J. Bone Miner. Res.* **8**: 707–717

Pitaru, S., Kotev-Emeth, S., Noff, D., Kaffuler, S., Savion, N. (1993) Effect of basic fibroblast growth factor on the growth and differentiation of adult stromal bone marrow cells: enhanced development of mineralized bone-like tissue in culture. *J. Bone Miner. Res.* **8**: 919–929

Raiche, A. T., Puleo, D. A. (2004) In vitro effects of combined and sequential delivery of two bone growth factors. *Biomaterials* **25**: 677–685

Reddi, A. H. (1992) Regulation of cartilage and bone differentiation by bone morphogenetic proteins. *Curr. Opin. Cell Biol.* **4**: 850–855

Renier, M. L., Kohn, D. H. (1997) Development and characterization of a biodegradable polyphosphate. *J. Biomed. Mater. Res.* **34**: 95–104

Richardson, T. P., Peters, M. C., Ennett, A. B., Mooney, D. J. (2001) Polymeric system for dual growth factor delivery. *Nat. Biotechnol.* **19**: 1029–1034

Ripamonti, U., Ramoshebi, L. N., Matsaba, T., Tasker, J., Crooks, J., Teare, J. (2001a) Bone induction by BMPs/OPs and related family members in primates. *J. Bone Joint Surg. Am.* **83-A** (Suppl. 1): S116–S127

Ripamonti, U., Crooks, J., Petit, J. C., Rueger, D. C. (2001b) Periodontal tissue regeneration by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (Papio ursinus). *Eur. J. Oral Sci.* **109**: 241–248

Roach, H. I., Baker, J. E., Clarke, N. M. (1998) Initiation of the bony epiphysis in long bones: chronology of interactions between the vascular system and the chondrocytes. *J. Bone Miner. Res.* **13**: 950–961

Rose, F. R. A. J., Oreffo, R. O. C. (2002) Bone tissue engineering: hope vs hype. *Biochem. Biophys. Res. Commun.* **292**: 1–7

Ruel-Gariepy, E., Chenite, A., Chaput, C., Guirguis, S., Leroux, J. C. (2000) Characterization of thermosensitive chitosan gels for the sustained delivery of drugs. *Int. J. Pharm.* **203**: 89–98

Saito, N., Okada, T., Toba, S., Miyamoto, S., Takaoka, K. (1999) New synthetic absorbable polymers as BMP carriers: plastic properties of poly-D,L-lactic acid-polyethylene glycol block copolymers. *J. Biomed. Mater. Res.* **47**: 104–110

Saito, N., Okada, T., Horiuchi, H., Murakami, N., Takakashi, J., Nawata, M., Ota, H., Miyamoto, S., Nozaki, K., Takaoka, K. (2001a) Biodegradable poly-D,L-lactic acid-polyethylene glycol block copolymers as a BMP delivery system for inducing bone. *J Bone Joint Surg Am* **83A**: S92–S98.

Saito, N., Okada, T., Horiuchi, H., Murakami, N., Takahashi, J., Nawata, M., Ota, H., Nozaki, K., Takaoka, K. (2001b) A biodegradable polymer as a cytokine delivery system for inducing bone formation. *Nat. Biotechnol.* **19**: 332–335

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

Saltzman, W. M., Olbricht, W. L. (2002) Building drug delivery into tissue engineering. *Nat. Rev. Drug Discov.* **1**: 177–186

Santos, E. M., Radin, S., Ducheyne, P. (1999) Sol-gel derived carrier for the controlled release of proteins. *Biomaterials* **20**: 1695–1700

Schmidmaier, G., Wildemann, B., Cromme, F., Kandziora, F., Haas, N. P., Raschke, M. (2002) Bone morphogenetic protein-2 coating of titanium implants increases biomechanical strength and accelerates bone remodelling in fracture treatment: a biomechanical and histological study in rats. *Bone* **30**: 816–822

Singh, M., Shirley, B., Bajwa, K., Samara, E., Hora, M., O'Hagan, D. (2001) Controlled release of recombinant insulin-like growth factor from a novel formulation of polylactide-co-glycolide microparticles. *J. Control. Release* **70**: 21–28

Sittinger, M., Bujia, J., Rotter, N., Reitzel, D., Minuth, W. W., Burmester, G. R. (1996) Tissue engineering and autologous transplant formation: practical approaches with resorbable biomaterials and new cell culture techniques. *Biomaterials* **17**: 237–242

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

Tabata, Y., Yamamoto, M., Ikada, Y. (1998) Comparison of release profiles of various growth factors from biodegradable carriers. In: *Biomaterials regulating cell function and tissue development*. Materials Research Society, Warrendale, pp 13–18

Takaoka, K., Koezuka, M., Nakahara, H. (1991) Telopeptide-depleted bovine skin collagen as a carrier for bone morphogenetic protein. *J. Orthop. Res.* **9**: 902–907

Tare, R. S., Oreffo, R. O., Clarke, N. M., Roach, H. I. (2002a) Pleiotrophin/osteoblast-stimulating factor 1: dissecting its diverse functions in bone formation. *J. Bone Miner. Res.* **17**: 2009–2020

Tare, R. S., Oreffo, R. O., Sato, K., Rauvala, H., Clarke, N. M., Roach, H. I. (2002b) Effects of targeted overexpression of pleiotrophin on postnatal bone development. *Biochem. Biophys. Res. Commun.* **298**: 324–332

Thompson, R. C., Yaszemski, M. J., Powers, J. M., Mikos, A. G. (1995) Fabrication of biodegradable polymer scaffolds to engineer trabecular bone. *J. Biomater. Sci. Polym. Ed.* **7**: 23–38

Ueda, H., Hong, L., Yamamoto, M., Shigeno, K., Inoue, M., Toba, T., Yoshitani, M., Nakamura, T., Tabata, Y., Shimizu, Y. (2002) Use of collagen sponge incorporating transforming growth factor-beta 1 to promote bone repair in skull defects in rabbits. *Biomaterials* **23**: 1003–1010

Uludag, H., D'Augusta, D., Palmer, R., Timony, G., Wozney, J. (1999a) Characterization of rhBMP-2 pharmacokinetics implanted with biomaterial carriers in the rat ectopic model. *J. Biomed. Mater. Res.* **46**: 193–202

Uludag, H., Friess, W., Williams, D., Porter, T., Timony, G., D'Augusta, D., Blake, C., Palmer, R., Biron, B., Wozney, J. (1999b) rhBMP-collagen sponges as osteoinductive devices: effects of *in vitro* sponge characteristics and protein pI on *in vivo* rhBMP pharmacokinetics. *Ann. N. Y. Acad. Sci.* **875**: 369–378

Uludag, H., D'Augusta, D., Golden, J., Li, J., Timony, G., Riedel, R., Wozney, J. M. (2000) Implantation of recombinant human bone morphogenetic proteins with biomaterial carriers: a correlation between protein pharmacokinetics and osteoinduction in the rat ectopic model. *J. Biomed. Mater. Res.* **50**: 227–238

Uludag, H., Gao, T. J., Porter, T. J., Friess, W., Wozney, J. M. (2001) Delivery systems for BMPs: Factors contributing to protein retention at an application site. *J. Bone Joint Surg. Am.* **83A**: S128–S135

Vacanti, C. A., Bonassar, L. J., Vacanti, J. P. (2000) Structural tissue engineering. In: Lanza, R. P., Langer, R., Vacanti, J. P. (eds) *Principles of tissue engineering*. Academic Press, New York

Vehof, J. W. M., Fisher, J. P., Dean, D., van der Waerden, J., Spauwen, P. H. M., Mikos, A. G., Jansen, J. A. (2002) Bone formation in transforming growth factor beta-1-coated porous poly(propylene fumarate) scaffolds. *J. Biomed. Mater. Res.* **60**: 241–251

Wang, D. S., Miura, M., Demura, H., Sato, K. (1997) Anabolic effects of 1,25-dihydroxyvitamin D3 on osteoblasts are enhanced by vascular endothelial growth factor produced by osteoblasts and by growth factors produced by endothelial cells. *Endocrinology* **138**: 2953–2962

Weber, F. E., Eyrich, G., Gratz, K. W., Maly, F. E., Sailer, H. F. (2002) Slow and continuous application of human recombinant bone morphogenetic protein via biodegradable poly(lactide-co-glycolide) foamspheres. *Int. J. Oral Maxillofac. Surg.* **31**: 60–65

Whang, K., Tsai, D. C., Nam, E. K., Aitken, M., Sprague, S. M., Patel, P. K., Healy, K. E. (1998) Ectopic bone formation via rhBMP-2 delivery from porous bioabsorbable polymer scaffolds. *J. Biomed. Mater. Res.* **42**: 491–499

Wheeler, D. I., Chamberland, D. I., Schmitt, J. M., Buck, D. C., Brekke, J. H., Hollinger, J. O., Joh, S. P., Suh, K. W. (1998) Radiomorphometry and biomechanical assessment of recombinant human bone morphogenetic protein 2 and polymer in rabbit radius ostectomy model. *J. Biomed. Mater. Res.* **43**: 365–373

Whitaker, M. J., Quirk, R. A., Howdle, S. M., Shakesheff, K. M. (2001) Growth factor release from tissue engineering scaffolds. *J. Pharm. Pharmacol.* **53**: 1427–1437

Winn, S. R., Uludag, H., Hollinger, J. O. (1998) Sustained release emphasizing recombinant human bone morphogenetic protein-2. *Adv. Drug. Deliv. Rev.* **31**: 303–318

Winn, S. R., Schmitt, J. M., Buck, D., Hu, Y. H., Grainger, D., Hollinger, J. O. (1999) Tissue-engineered bone biomimetic to regenerate calvarial critical-sized defects in athymic rats. *J. Biomed. Mater. Res.* **45**: 414–421

Woo, B. H., Fink, B. F., Page, R., Schrier, J. A., Jo, Y. W., Jiang, G., DeLuca, M., Vasconez, H. C., DeLuca, P. P. (2001) Enhancement of bone growth by sustained delivery of recombinant human bone morphogenetic protein-2 in a polymeric matrix. *Pharm. Res.* **18**: 1747–1753

Wozney, J. M., Rosen, V. (1998) Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. *Clin. Orthop.* **346**: 26–37

Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M., Wang, E. A. (1988) Novel regulators of bone formation: molecular clones and activities. *Science* **242**: 1528–1534

Xiong, Z., Yan, Y. N., Zhang, R. J., Sun, L. (2001) Fabrication of porous poly(L-lactic acid) scaffolds for bone tissue engineering via precise extrusion. *Scripta Mater.* **45**: 773–779

Yamamoto, M., Tabata, Y., Ikada, Y. (1998) Ectopic bone formation induced by biodegradable hydrogels incorporating bone morphogenetic protein. *J. Biomater. Sci. Polym. Ed.* **9**: 439–458

Yamamoto, M., Tabata, Y., Hong, L., Miyamoto, S., Hashimoto, N., Ikada, Y. (2000) Bone regeneration by transforming growth factor beta 1 released from a biodegradable hydrogel. *J. Control. Release* **64**: 133–142

Yamamoto, M., Takahashi, Y., Tabata, Y. (2003) Controlled release by biodegradable hydrogels enhances the ectopic bone formation of bone morphogenetic protein. *Biomaterials* **24**: 4375–4383

Yang, X., Tare, R. S., Partridge, K. A., Roach, H. I., Clarke, N. M., Howdle, S. M., Shakesheff, K. M., Oreffo, R. O. (2003a) 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, X. B., Whitaker, M. J., Clarke, N. M. P., Sebald, W., Howdle, S. M., Shakesheff, K. M., Oreffo, R. O. C. (2003b) *In vivo* human bone and cartilage formation using porous polymer scaffolds encapsulated with bone morphogenetic protein-2. *J. Bone Miner. Res.* **18**: 1366

Yang, X. B., Green, D. W., Roach, H. I., Clarke, N. M. P., Anderson, H. C., Howdle, S. M., Shakesheff, K. M., Oreffo, R. O. C. (2003c) Novel osteoinductive biomimetic scaffolds stimulate human osteoprogenitor activity – implications for skeletal repair. *Connect. Tissue Res.* **44**: 312–317

Yasko, A. W., Lane, J. M., Fellinger, E. J., Rosen, V., Wozney, J. M., Wang, E. A. (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

Zellin, G. (1998) Growth factors and bone regeneration. Implications of barrier membranes. *Swed. Dent. J.* **129**: 7–65

Zellin, G., Linde, A. (1997) Treatment of segmental defects in long bones using osteopromotive membranes and recombinant human bone morphogenetic protein-2. An experimental study in rabbits. *Scand. J. Plast. Reconst. Surg. Hand Surg.* **31**: 97–104

Zhao, G., Monier-Faugere, M. C., Langub, M. C., Geng, Z., Nakayama, T., Pike, J. W., Chernausek, S. D., Rosen, C. J., Donahue, L. R., Malluche, H. H., Fagin, J. A., Clemens, T. L. (2000) Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. *Endocrinology* **141**: 2674–2682

Ziegler, J., Mayr-Wohlfart, U., Kessler, S., Breitig, D., Gunther, K. P. (2002) Adsorption and release properties of growth factors from biodegradable implants. *J. Biomed. Mater. Res.* **59**: 422–428