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Review article

Localized delivery of growth factors for bone repair

Vera Luginbuehl, Lorenz Meinel, Hans P. Merkle, Bruno Gander*

Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology Zurich (ETH Zurich), Zurich, Switzerland

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Abstract

Delivery of growth factors for tissue (e.g. bone, cartilage) or cell repair (e.g. nerves) is about to gain important potential as a future therapeutic tool. Depending on the targeted cell type and its state of differentiation, growth factors can activate or regulate a variety of cellular functions. Therefore, strictly localized delivery regimens at well-defined kinetics appear to be logical prerequisites to assure safe and efficacious therapeutic use of such factors and avoid unwanted side effects and toxicity, a major hurdle in the clinical development of growth factor therapies so far. This review summarizes various approaches for localized growth factor delivery as focused on bone repair. Similar considerations may apply to other growth factors and therapeutic indications. Considering the vast number of preclinical studies reported in the area of growth factor-assisted bone repair, it surprises though that only two medical products for bone repair have so far been commercialized, both consisting of a collagen matrix impregnated with a bone morphogenetic protein. The marked diversity of the reported growth factors, delivery concepts and not yet standardized animal models adds to the complexity to learn from past preclinical studies presented in the literature. Nonetheless, it is now firmly established from the available information that the type, dose and delivery kinetics of growth factors all play a decisive role for the therapeutic success of any such approach. Very likely, all of these parameters have to be adapted and optimized for each animal model or clinical case. In the future, systems for localized growth factor delivery thus need to be designed in such a way that their modular components are readily adaptable to the individual pathology. To make such customized systems feasible, close cooperative networks of biomedical and biomaterials engineers, pharmaceutical scientists, chemists, biologists and clinicians need to be established.

Keywords: Growth factors; Bone repair; Drug delivery; Localized delivery; Controlled delivery; Release kinetics; Dose effects; Clinical safety

1. Introduction

Bone repair is a complex cascade of biological events regulated by specific cells, the extracellular matrix (ECM) and distinct growth factors [1]. Bone tissue has the capacity of postnatal self-reconstruction. In combination with their binding proteins, growth factors form physiological depots in the bone matrix, from which they are released and act as local regulators of cell function. They stimulate cellular differentiation, proliferation, migration, adhesion, and gene expression. They act by binding to the extracellular domain of a target growth factor receptor, thus activating the intracellular signal-transduction, which ultimately reaches the nucleus and results in the transcription of mRNA and the synthesis of the respective protein(s) [2–4]. However, in severe pathological situations such as complicated fractures, trauma, treatment of bone tumors, joint replacement, congenital defects or spinal fusion, the damaged bone will not form or regenerate spontaneously. To restore structural and functional integrity, either autografts or allogenic bone from tissue banks are required, even though severe adverse effects and morbidity are associated with these techniques [5]. Biochemical stimulation of local bone healing via the delivery of growth factors can supplement conventional bone repair therapies [6–8]. Growth factor effects are pleiotropic, and several isoforms of the same growth factor may bind to a single receptor. Also, they show redundancy, i.e. different receptors may be activated by a single ligand. The short biological half-life, the lack of long-term stability

Abbreviations: aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; BSA, bovine serum albumin; ECM, extracellular matrix; FDA, Food and Drug Administration; HBGF, heparin-binding growth factor; IGF, insulin-like growth factor; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PEG, polyethyleneglycol; pI, isoelectic point; PLA, poly(D,L-lactide); PLGA, poly(D,L-lactide-co-glycolide); TCP, tricalcium phosphate; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

* Corresponding author. Drug Formulation and Delivery Group, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology Zurich (ETH Zurich), Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. Tel.: +41-1-635-6012; fax: +41-1-635-6881.

E-mail address: bruno.gander@pharma.ethz.ch (B. Gander).

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and tissue-selectivity of growth factors, their potential toxicity and risk of cancerogenic activity demand for controlled delivery systems for therapeutic applications [9]. There are three different delivery approaches: (i) systemic administration of growth factors, (ii) their localized delivery by incorporation in a carrier matrix, and (iii) gene therapy. This review focuses on localized protein delivery for bone repair. This area is considered to be one of the imminent fields for clinical usage of growth factors in the near future with great impact in bone tissue engineering [10]. For 30 years, the localized application of growth factor delivery systems has been intensively investigated in orthopaedic research [11]. The consolidated findings from this research may be adaptable to other pathologies and therapeutic indications. In this review we describe typical difficulties associated with growth factor therapy and present an overview of selected preclinical studies, followed by a conceptual description of both established and proposed delivery strategies meeting orthopaedic needs. We describe the prime importance of customized and optimized delivery systems for clinical success, as they are currently envisaged. Pharmacokinetics, dosing issues, safety and efficacy problems are further aspects that will be given adequate and critical attention in this review.

2. Difficulties in growth factor therapy

2.1. Materials and animal models

In 1965, Marshall Urist discovered the osteoinductive capacity of demineralized bone matrix [12]. Since then, many growth factors have been isolated. Thanks to the advances in recombinant DNA technology, sufficient quantities of these factors in pharmaceutical quality have become available for therapeutic use. The most important growth factors with potential for bone regeneration encompass the various bone morphogenetic proteins (BMP), transforming growth factor beta (TGF-β), insulin-like growth factors (IGF), fibroblast growth factors (FGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). A detailed description of their biological and clinical roles in development and repair of the skeleton is available [13]. A major problem for their clinical use is the need for development of appropriate delivery systems. Growth factor delivery has been studied with diverse platform technologies and materials, in different bone defects and various animal models (Table 1). Most trials have been performed with growth factors from the BMP family, mainly BMP-2 and BMP-7. Two BMP-containing products based on a collagen sponge have recently obtained approval by several federal agencies for the treatment of long bone fracture non-unions and lumbar interbody fusion [14]. Besides these two products, registered for specialized indications, no other growth factor formulation for the enhancement of bone repair is at present commercially available.

Various medical devices made from collagen, hyaluronan, chitosan, fibrin, silk, and synthetic polymers, ceramics and injectable calcium phosphate cements, have been tested as alternatives to autogenous bone grafts [15–18]. The materials have been molded into different geometries and configurations; i.e. membranes, granules, matrices, implant coatings, microparticles, hydrogels, and foams.

The animal models used were as diverse as the materials and devices. Many animal species, from mice to monkeys,

Table 1
Selected experimental studies of localized bone growth factor delivery with respect to carrier material, animal model, and type of restored bone

Growth factor	Carrier (category)	Species	Model	Bone type	Ref.
BMP-2	Autogenic graft (c)	Human	Tibia noniunion	Long bone	[112]
BMP-2	Collagen (n)	Goat	Closed tibia fracture	Long bone	[113]
BMP-2	PLA coating (s)	Sheep	Spine fusion	Spinal bone	[114]
BMP-7	Collagen (n)	Baboon	Calvarial defect	Skull	[115]
BMP-3	Hydroxyapatite/TCP (i)	Rat	Segmental femoral defect	Long bone	[116]
BMP-13	Collagen (n)	Rat	Intramuscular, tendon defect		[117]
TGF-β1	Demineralized bone matrix +carboxymethyl cellulose (c)	Rabbit	Calvarial defect	Skull	[118]
TGF-β1	TCP + bone marrow (c)	Rabbit	Radius defect	Long bone	[119]
TGF-β2	BSA-solution	Rabbit	Tibia fracture	Long bone	[120]
TGF-β3	β-TCP (i)	Baboon	Spine defect	Spinal bone	[121]
IGF-I	Mini osmotic pump	Rat	Calvarial defect	Skull	[122]
IGF-I	PLGA microparticles (s)	Sheep	Tibia defect, diaphyseal defect	Long bone	[67]
IGF-I	PLA coating (s)	Minipig	Tibia defect	Long bone	[123]
IGF-II	Collagen (n)	Rat	Facial defect	Skull	[124]
bFGF	Hyaluronan gel (n)	Baboon	Fibula defect	Long bone	[125]
bFGF	Gelatin hydrogel (n)	Dog	Maxillary furcation defect	Mandibular bone	[126]
aFGF	Agarose (n)	Rat	Calvarial defect	Skull	[127]
PDGF BB	Collagen (n)	Rabbit	Tibia defect	Long bone	[128]
PDGF BB	Chitosan/TCP (c)	Rat	Calvarial defect	Skull	[129]

Categories of carrier material: (i) inorganic material; (s) synthetic polymers; (n) natural polymers; (c) composites.

and a variety of musculoskeletal models, such as critical and non-critical size cranial or long bone defects, and several vertebrae approaches have been used. Small animal bone physiology is difficult to compare with that of larger species such as sheep, goat or primates, and extrapolation to the human disease situation is highly disputable [19]. Hence, it may be inappropriate to compare data from different studies, because physicochemical carrier properties, the type of growth factor and its delivery, the complex biological interaction between different growth factors, and the diverse species and defect models strongly determine the preclinical and clinical result. The appropriate choice of the delivery system for a particular growth factor is essential to induce a specific biological effect, as demonstrated by several examples in the literature, where failure of bone repair was associated with the type of delivery device. For example, locally administered solutions of bFGF did not promote bone regeneration in rabbit skull defects in contrast to bFGF incorporated in gelatin hydrogels [20]. Failure of BMP in an insoluble bone matrix to induce bone growth around titanium implants was attributed to unsuitable carrier properties [21]. Finally, locally applied IGF-I failed to induce bone repair when delivered by an osmotic pump directly into the osteotomy [22,23], but was successful when embedded in biodegradable microspheres to heal segmental long bone defects [24]. Consequently, several critical issues need to be addressed when selecting or designing a delivery system for growth factors, as exemplified in the following for bone repair.

2.2. Requirements for bone growth factor delivery systems

A delivery system designed for bone repair ideally combines osteoconductive and osteoinductive properties, in a way that new bone formation can be enhanced through an adequately shaped 3D-scaffold (osteoconduction) and by a biological stimulus (osteoinduction). The delivery device should (i) provide a time and dose-controlled release of the bioactive growth factor, (ii) offer a scaffold that enhances cell recruitment and attachment, and (iii) allocate void space to promote cell migration and angiogenesis. Additional requirements for a carrier include high biocompatibility, adequate biodegradability, mechanical congruency, low toxicity, malleability, ease of manufacture (reproducibility, scaling-up), and cost effectiveness [25]. For implantation of a delivery system, the anatomic location of the therapeutic intervention, the vitality of the adjacent bone and surrounding soft-tissue, and the environmental mechanical stress induced by the reconstructive system have to be taken into account. Particular challenges for developing an optimal delivery system encompass the achievement of sufficient mechanical implant stability, especially in weight-bearing bones, while offering a porous structure for cell ingrowth and angiogenesis; further, the carrier should degrade within a few weeks to months, to minimize interference with the normal healing process, but maintain optimal factor retention. Clearly, there is no ideal carrier or delivery system for all growth factors or pathologies. Optimizing and customizing currently known carriers require continued attention and will then hopefully lead to more efficient therapies for musculoskeletal disorders.

3. Delivery strategies and systems

3.1. Non-covalent growth factor immobilization

For localized growth factor delivery, proteins are most commonly immobilized through non-covalent or covalent binding to a carrier matrix. Non-covalent binding includes physical entrapment, adsorption or ionic complexation. The first controlled delivery systems were of reservoir type, where osmotic pressure combined with polymer membranes regulated the rate of drug release (Fig. 1A) [26]. However, the use of such systems is limited, because of difficulties in controlling the release of large molecules, and because of problems related to dose dumping. The discovery that hydrophobic polymer matrices can release physically entrapped proteins over extended periods of time [27] resulted in the development of a variety of delivery systems. Bone growth factors have been physically entrapped in polymeric microparticles, liposomes, hydrogels, foams or bone cements (Fig. 1B). Growth factors have also been dispersed in various types of materials, used to coat implant surfaces (Fig. 1C). Titanium implants, for example, have been coated with poly(D,L-lactide) in which TGF-β1 and IGF-I were embedded [28]. Besides physical entrapment, physical adsorption or physisorption of proteins onto implant materials has been frequently used (Fig. 1D). For example, the impregnation of a preformed absorbable collagen sponge with BMP solutions is a commonly used technique to fabricate BMP delivery devices [29]. Even though passive adsorption methods greatly benefit from their simplicity, conformational changes and denaturation are widespread leading to loss of protein activity as well as irreversible binding of growth factors. For example, 5–10% of an implanted dose of rhBMP-2 was irreversibly bound to a mineral-based carrier without further release [30]. An interesting approach to passive protein adsorption is to exploit the natural binding properties of certain growth factors to components of the ECM (Fig. 1E). Such naturally binding growth factors are bFGF, TGF-β and BMP-2, all characterized by their high affinity for heparin, thus termed heparin-binding growth factors (HBGF) [31]. Carriers prepared from synthetic heparin-like polymers or from natural matrix materials such as fibrinogen, collagen or gelatin in combination with heparin sulfate, bind specifically HBGFs and protect them from proteolytic inactivation and denaturation [32]. In absence of prior binding to heparan sulfate, FGF does

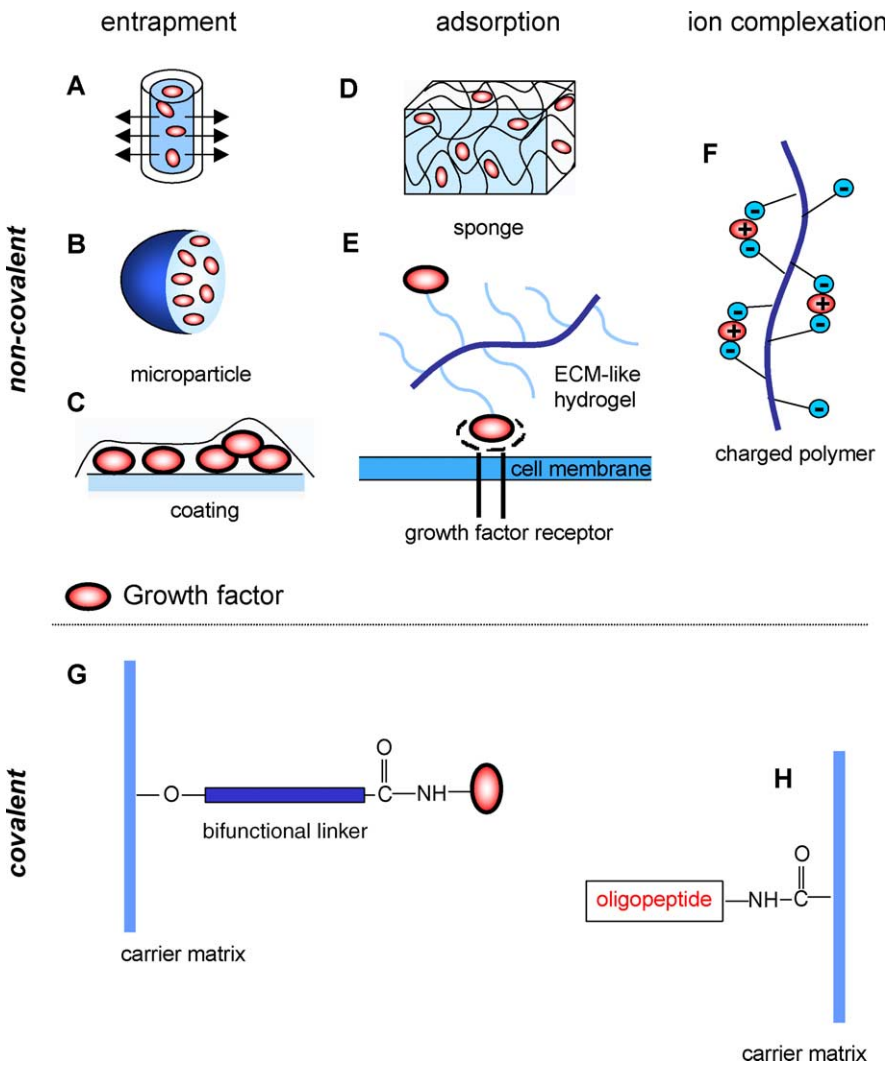

Fig. 1. Growth factor delivery systems and strategies for bone repair, encompassing non-covalent (A–F) and covalent (G, H) growth factor entrapment. (A–C) Physically entrapped growth factors in a reservoir device (A), a microparticle (B), or polymeric coating implant (C), which can be released by diffusion through a polymeric matrix or membrane, with or without concomitant bioerosion of the delivery system. (D) Adsorption of growth factors through physico-chemical interactions with sponge material, e.g. collagen; release occurs upon desorption, which is highly sensitive to the environmental conditions. (E) Heparin-binding growth factors bound to heparan sulfate-substituted proteoglycans from the extracellular matrix are favorably presented to their receptors. (F) Ionic complexation of growth factors with oppositely charged macromolecules, mostly derived from natural polymers; release occurs by ion exchange mechanism, which is highly sensitive to the environmental conditions. (G, H) Covalently bound growth factors attached via bifunctional linker (G) or by direct coupling of growth factor derived oligopeptide (H) to the carrier matrix.

not bind to its cellular receptor and, consequently, cannot exert its mitogenic activity [33]. This example demonstrates that harmonizing a biomaterial with the physico-chemical and biological properties of growth factors can potentiate their biological effects. A third possibility of non-covalent association of a protein with a biomaterial is by ion complexation (Fig. 1F). Proteins with different isoelectric points (pI) may be used for polyion complexation with charged macromolecules, such as alginate, chitosan, gelatin, hyaluronan and also synthetic polyelectrolytes [34–36]. As with passive adsorption, problems related to irreversible ion complexion can cause protein inactivation. This was reported for the cationic protein TGF-β1, which forms an irreversible coacervate with the anionic alginate [37]. To prevent inactivation of proteins upon complexation with polyelectrolytes, additives may be necessary, e.g. poly(acrylic acid) shielded TGF-β1 from the harmful effect of its interaction with alginate [37]. Depending on the characteristics of both the growth factor and the biomaterial, protein–polymer interactions may not be exclusively electrostatic, but also hydrophobic.

An alternative to non-covalent binding is the crystallization of growth factors. We investigated the crystallization of TGF-β3 in the presence of dioxane [38–40]. The resulting crystals contain one dioxane per protein in an internal hydrophobic pocket, thus promoting three crystal contact interfaces per molecule instead of one.

This may explain why the resulting crystals were mechanically robust and suitable for formulation and processing. Further, the crystals demonstrated low aqueous solubility and dissolution rate, making them attractive for sustained release purposes. Importantly, the dioxane-cocrystallized TGF-β3 retained full bioactivity as demonstrated in a cell culture model.

3.2. Covalent growth factor immobilization

Covalent immobilization is another strategy to retain growth factors for longer periods of time at the delivery site. Covalent immobilization of growth factors was not a priori expected to maintain biological activity, because it may negatively affect their binding to the receptors and the subsequent dimerization of the receptors in the plane of the membrane. Nevertheless, if appropriately designed, conjugated growth factors, so called tethered growth factors, offer important control of the amount and distribution of these components in solid matrices and facilitate the establishment of growth factor gradients [41,42]. Bentz et al. [43] covalently cross-linked TGF-β2 via activated polyethyleneglycol to fibrillar collagen and showed in vitro and in vivo activities (Fig. 1G). TGF-β1 covalently tethered to a polymer scaffold retained its activity to increase ECM production of rat vascular smooth muscle cells [44]. A BMP-2 derived oligopeptide linked directly to an alginate gel induced ectopic bone formation in rat muscle (Fig. 1H) [45]. These case studies suggest the feasibility of covalent growth factor linkage to different matrices, even though maintenance of biological activity is a critical aspect and needs to be evaluated carefully on a case-by-case basis. Presentation of tethered growth factors may help to expedite their clinical use by permitting greater control of temporal and spatial availability in the extracellular environment.

3.3. Chemical modifications of bone growth factors

Besides growth factor immobilization, protein modifications allow for changes in carrier affinity, bioactivity, stability and bioavailability (Table 2). To modulate changes in carrier affinity, succinylated [30], acetylated [46] or biotinylated [47] BMP derivatives have been produced. Another approach to change the affinity to a carrier material is by truncation of the growth factor, e.g. by plasmin cleavage [17]. Plasmin cleavage of rhBMP-2 removed a positively charged fraction of the N-terminus yielding a protein with a lowered pI-value and, therefore, reduced electrostatic interaction with the negatively charged proteoglycans of the ECM [48]. Heterodimers of various BMPs were genetically engineered with the objective of improving their bioactivity relative to BMP homodimers [49,50]. The *Xenopus* BMP-4/7 heterodimer indeed showed a 20-fold higher bone-inducing capacity than the homodimers of xBMP-4 and xBMP-7, or as mixtures of these homodimers [49]. A TGF-β1 fusion protein bearing a collagen binding domain was engineered to selectively target collagen type I and to afford slow release of the growth factor [51]. Another fusion protein, aFGF conjugated with heparan sulfate proteoglycan, was constructed to protect aFGF from proteolytic degradation [52]. The development of peptide mimicry of growth factors provides an alternative system for local growth factor delivery. For example, a BMP-2-derived oligopeptide covalently coupled to alginate hydrogel induced ectopic bone formation in rats [45]. All of these examples show that protein modifications are suitable to modulate intrinsic protein properties or protein–carrier interactions. Genetically engineered fusion proteins with specific binding domains for the ECM are particularly attractive for bone targeting.

Table 2
Chemical modifications of bone growth factors as delivery strategies

Modification	Growth factor	System	Delivery strategy	Ref.
Derivation	BMP-2	Succinylation, acetylation	Change in carrier affinity due to pI shift, increase in solubility binding to biomaterial	[30,46]
		Biotinylation		[47]
Dimerization	BMP-2, 4, 6,7	Genetically engineered BMP-4/7, 2/7, 2/6 heterodimers	Dose reduction, improvement of osteogenic signal	[49,50]
Fusion proteins	TGF-β1	Genetically engineered rhTGF-β1 fusion protein bearing a von Willebrand's factor derived collagen binding domain	Targeting collagen type I, sustained release	[51]
	aFGF	Genetically engineered fusion protein of aFGF and heparan sulfate proteoglycan core binding protein	Manifestation of heparin-independent biological activity, protection from proteolytic degradation	[52]
Oligopeptides	BMP-2	BMP-2 derived oligopeptide covalently linked to alginate	Improvement of stability and activity, reduction of burst effect	[45]
Enzymatic cleavage	BMP-2	Plasmin cleaved rhBMP-2	Possible reduction of non-specific interactions with the extracellular matrix, enhanced in vitro activity (not confirmed in vivo)	[30,130]

4. Effects of release kinetics

4.1. Experimental release kinetic studies

There are three phases in physiological bone repair, i.e. the inflammatory, chondrogenic and osteogenic phases. In each of these phases the expression of growth factors follows specific kinetics [53]. This was demonstrated at the mRNA and corresponding protein levels in diverse fracture models by in situ hybridization, polymerase chain reaction and immunohistochemistry [54,55]. Some factors such as PDGF and BMP-2 are predominantly expressed during the early inflammatory phase, others are up-regulated during the chondrogenic and osteogenic phases, have a biphasic expression pattern or are constitutively present [12]. Not only growth factor concentrations change in a time-dependent manner, but also the expression of their receptors. The space- and time-restricted expression patterns of growth factors suggest specific functional roles in the repair process. Furthermore, the cell pool present in the defect zone is dynamic by nature. Different stimuli can attract different cell types to invade the compromised area, and certain cells capable to undergo differentiation change their phenotype along with the progressing healing events. Therefore, the impact of local release kinetics for the therapeutic enhancement of skeletal repair becomes evident. The importance of release kinetics for growth factor-based therapies and tissue engineering has been suggested [56,57]. However, very few have investigated the influence of release kinetics on bone regeneration or optimized delivery systems for growth factor release. At the cellular level, the effect of bFGF and TGF-β1 release kinetics has been investigated by Dinbergs et al. [58]. They found that sustained release of bFGF was more potent than bolus administration for vascular endothelial and smooth muscle cell proliferation, while the reverse was true for TGF-β1. Talwar et al. [59] examined the effect of rhBMP-2 release from slow and fast degrading gelatin carriers in rat periodontal defects. No significant differences on new bone formation between the slow and the fast degrading carriers were found. The impact of local BMP-2 pharmacokinetics on osteoinductivity was also addressed in another BMP study [60], where growth factor retention in collagen implants was modulated by using rhBMP-2, rhBMP-4 and plasmin-cleaved rhBMP-2, all having different pI-values (around 9, 5–7 and 6, respectively) and, therefore, different affinities to the carrier matrix. Radioactivity counts in the explants indicated that rhBMPs with lower pI were retained to a lesser extent at the implant site. rhBMPs with prolonged retention time were more osteoinductive in the rat ectopic assay. Our own unpublished results suggest that distinct IGF-I release profiles (linear, pulsatile and slow releasing), obtained with poly(lactic-co-glycolic acid) (PLGA) and poly(lactic acid) (PLA) microspheres, significantly influenced the progress of bone repair in drill hole defects in sheep.

While timing of drug release is important, the dynamic nature of the healing zone makes it difficult to assess the state of the defect. It is certainly dependent on the type of fracture, its location and appearance, the patient's age, sex, hormone and nutritional status, illness and other parameters. Thus, individualized or customized kinetics that respond specifically to the actual pathological situation could help to overcome these limitations. Further investigation is required to clarify whether mimicking natural expression patterns of growth factors by adequate release kinetics is advantageous for bone healing. Nonetheless, the aforementioned examples suggest that optimized growth factor release kinetics can significantly improve therapeutic responses.

4.2. Control of growth factor release

The tight control of growth factor release according to a predetermined profile may be critical for the design of delivery devices. Depending on the device's geometry, volume, porosity, hydrophobicity and biodegradation, and its affinity to the particular growth factor and site of implantation, growth factor release from a formulation can either be (i) diffusion-controlled, (ii) chemical and/or enzymatic reaction-controlled, (iii) solvent-controlled [61], or (iv) controlled by combinations of these mechanisms. Diffusion-controlled release is governed by the solubility and diffusion coefficient of the protein in the aqueous medium, protein partitioning between the aqueous medium and material of the device, the protein loading and the diffusional distance [62]. An example of diffusion-controlled release is rhBMP-2 release from porous PLGA scaffolds that was regulated via adjustment of the median pore size ranging from 7 to 70 μm [63]; another way of rate control is exemplified by TGF-β1 release from coral particles that was modulated through modification of adsorption conditions (pH 3, 7.4 and 11, with 0.1% BSA or gelatin) and particle size [64]. Chemical and/or enzymatic reaction-controlled systems include erodible systems, where the protein is physically immobilized in the carrier matrix and released by degradation or dissolution of the carrier, or systems, where the protein is chemically bound to the polymer backbone and released upon hydrolytic or enzymatic cleavage of the bond. Varying the degree of cross-linking in hydrogels is one possibility to modify carrier degradation time and, thereby, control growth factor release. As an example, TGF-β1 release from cross-linked collagen sponges depended on the extent of cross-linking, as observed after subcutaneous implantation into mice backs [65]. In solvent-controlled or swelling-controlled systems, the protein is embedded in a carrier matrix and diffusional release occurs as a consequence of the rate-controlled penetration of solvent (water) into the device. Hence, when it comes to mimic the space- and time-restricted physiologic pattern of growth factor

kinetics by the local release kinetics of an implant, different strategies of controlling growth factor delivery are available.

5. Dose effects

Growth factor effects are dose-dependent. For example, Zellin et al. [66] showed dose-dependent bone healing of rat mandibular defects with rhTGF-β1, irrespective of the carrier type, which were a methylcellulose gel, a porous coral composite, and PLGA particles. In our own experiments, IGF-I-loaded PLGA microspheres, administered into diaphyseal drill hole defects in sheep, promoted new bone formation in a dose-dependent fashion for doses of 30 and 80 μg, but not for 100 μg IGF-I, which resulted in roughly the same effect as upon 80 μg [67,68]. In contrast, 48 ng IGF-I adsorbed on TCP cylinders stimulated bone turnover and ceramic resorption, but did not promote osteogenesis in rabbit femoral defects [69]. Therefore, minimally effective doses need to be determined, but above a certain threshold, bone formation cannot be further enhanced. The application of excessive doses can provoke adverse effects or inhibit bone formation, as reported by Aspenberg et al. [70]. Increasing TGF-β1 doses adsorbed on hydroxyapatite reduced bone ingrowth into titanium bone chambers in a rat model. It is noteworthy that the required growth factor dose regimen is also model-dependent. A given dose of BMP-2 embedded in a collagen carrier diminished bone ingrowth into a titanium chamber implanted in bone, while the same dose promoted bone ingrowth when the chamber was placed at an intramuscular site in rabbits [71]. Further, a species-specific dose response was observed in preclinical studies using rhBMP-2. Osteoinduction was observed at concentrations (expressed as micrograms of rhBMP-2 per unit volume of the matrix) of 25 μg/ml in rodents to 50 μg/ml in dogs, 100 μg/ml in non-human primates and 800 μg/ml in humans [72]. In contrast, the physiologic concentration of BMP-2 in normal bone is approximately 2 ng/g, which is sufficient for bone healing [73]. For most growth factors, administration of supraphysiologic doses is generally necessary [74]. The need for supraphysiologic concentrations seems to be related to inappropriate delivery kinetics, especially a too short maintenance of physiologic levels of growth factors [74]. Supraphysiologic concentrations may also be required to overcome the effects of natural inhibitors of growth factors [75]. Further complications in human clinical settings are genetic background, lifestyle, physical activity and age of the patients as well as variable pathology and additional medications, which all may affect the required dose. In conclusion, successful growth factor delivery requires dosage customization for each factor and delivery system, preclinical model and clinical case.

6. Safety and efficacy

Safety and efficacy of growth factor therapies can be considerably enhanced by the use of appropriate delivery systems. Akamaru et al. [76] showed that simple carrier matrix modifications consisting of the addition of ceramic phosphate granules can enhance the delivery of BMP-2 in spine fusion, thereby improving the efficacy of this therapy. Evidently, the materials used to prepare the delivery system should themselves be non-toxic, well standardized and generally completely resorbable without residues causing a host response [77]. Adverse effects have been mainly associated with systemic growth factor administration, whereas localized delivery is significantly safer. Nonetheless, high doses of locally administered rhBMP-2 caused heterotopic bone and bone-cyst formation during defect healing in dogs [78]. Ectopic bone formation is a considerable problem with the use of BMPs, because these factors can induce bone formation also in non-bony tissues. Oedema formation was reported in rabbits after subperiostal injections of TGF-β2 into connective tissues [79]. Accurate growth factor localization is therefore pivotal. The primary role of a delivery system for bone repair is to maintain the factor at the site of implantation and retain the drug from excessive initial dose dumping (burst release). Other safety issues associated with the use of growth factors in bone encompass the risks of bony overgrowth, immune responses and osteoclastic activation [80]. Stimulatory growth factor effects on osteoclasts (bone resorbing cells) have only been reported in vitro [81,82], but osteoclast stimulation may cause bone resorption also in vivo, especially under high dose regimens. In the large number of preclinical and clinical studies reported, local adverse effects caused by growth factor interventions were rare [83,84]. However, proper clinical safety studies in humans have been performed only with BMP-2 and BMP-7. Little is also known about individual variations of responsiveness to growth factor treatment. Thus, the incidence of adverse events upon growth factor therapy must be analyzed carefully, and balanced against accepted benefits.

The variety of the so far used animal models, dose regimens, delivery systems and growth factors complicates an objective evaluation of the efficacy of such systems (see also Table 1). Future investigations should preferably follow commonly recognized and standardized protocols, and the experimental models should mimic specific clinical problems. Very importantly, a control group treated with autologous grafts, which is the clinical gold standard, should be included as well as long-term observations of the healing fracture. Randomized double-blinded studies are required for human clinical trials. Finally, one should always keep in mind that a therapy might be inefficacious because of too low or too high doses, inappropriate delivery systems with inadequate release kinetics or lost activity, or because a given growth factor is pharmacologically inactive in a given animal model or clinical situation.

7. Future perspectives

7.1. Genomic and proteomic approaches

Future growth factor delivery systems must be further improved with regard to release control, dosing, efficacy and safety. For this purpose, more sophisticated delivery devices have to be developed in the future. A better understanding of the molecular events and mechanisms regulating bone repair and remodeling may improve current treatments [85]. For this aim, genomic and proteomic approaches to identify key markers for the related transcriptional and translational shifts involved in cell differentiation, cell proliferation and skeletal development will be quite useful [86,87]. These approaches have been used to investigate genetic profiles of osteoblast differentiation in murine MC3T3-E1 cell lines [88,89], calvaria cells [90] and immortalized adult human osteoblasts [91]. Using microarry analysis, de Jong et al. [92] investigated selective gene induction by BMP-2, TGF-β and activin A in relation to their capacity to control differentiation of mesenchymal precursor cells C2C12 into osteoblastic cells. The quantification of expression profiles of in concert acting growth factors could serve as an optimal blueprint to establish optimal release kinetics. Genomic and proteomic approaches may be equally useful as analytical tools, e.g. to monitor changes in gene and protein expression in response to the delivery of a given growth factor, a specific delivery regimen, the growth factor's release kinetics or the duration of its release pulse. The resulting information could provide an appealing rationale for the selection of suitable delivery technologies or the customization of the delivery regimen.

7.2. Intelligent drug delivery systems

The ideal delivery system should provide growth factors in response to physiological requirements, having the capacity to 'sense' changes of the bone defect's microenvironment and alter growth factor release accordingly. Intelligent polymers that can respond to a variety of physical, chemical and biological stimuli have great potential for the design of closed-loop drug delivery systems [93,94], in which growth factor delivery is self-regulated in response to a specific stimulus, and natural feedback mechanisms can be mimicked. An example represents the enzyme-sensitive delivery of rhBMP-2 [95]. Here, Hubbell and coworkers aimed at the molecular engineering of gels that were loaded with rhBMP-2, decorated with ligated integrin ligands and cross-linked with bis-cysteine peptides that were substrates of matrix metalloproteinases (MMP) (Fig. 2). Primary human fibroblasts attached to the integrin-binding ligands. Cell surface-associated MMPs cleaved proteolytically the gels so that rhBMP-2 was released. This process depended on the MMP substrate affinity, adhesion ligand concentration, and network cross-linking density. In fact, suitable gels to treat critical defects in rat cranium were completely infiltrated by cells and remodeled into bony tissue within 4 weeks at a dose of 5 μg per defect. However, MMP activity is not necessarily related to physiological signals of BMP-2 up-regulation and induction of new bone formation. The proof-of-concept of the therapeutic applicability and benefit of this approach needs to be demonstrated by both pre-clinical and clinical studies. Such intelligent drug delivery systems may represent a step towards individualizing release kinetics.

7.3. Multiple growth factor delivery

Since growth factors act in a coordinated cascade of events to restore bone, delivering combinations of growth factors may have great potential. Treatment with growth factor combinations exhibited both stimulatory and inhibitory responses on bone formation. For example, the combined application of IGF-I and TGF-β1 showed a synergistic effect on fracture healing in a rat tibia fracture model [96], whereas the combination of BMP-2 and bFGF

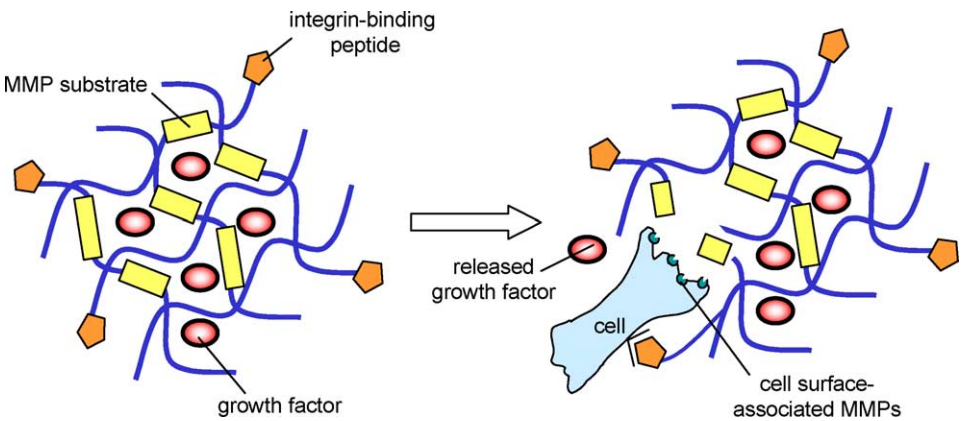

Fig. 2. Enzyme-sensitive rhBMP-2 drug delivery system adapted from Hubbell and coworkers [95,131]. Branched PEGs are functionalized with integrin-binding peptides and cross-linked via bis-cysteine MMP substrate peptides. Cell infiltration of the gels is enhanced by the interaction of cell adhesion receptors and bound integrin-binding peptides. Physically entrapped rhBMP-2 is mainly released upon enzymatic cleavage of the cross-linking peptides by cell-membrane associated MMPs.

absorbed to a collagen sponge resulted in decreased bone formation, also in a rabbit tibia fracture model [97]. These studies demonstrate that growth factor combinations have to be chosen carefully, and that adequate release kinetics might be critical for successful bone healing. Mimicking natural expression patterns for each growth factor may be one approach to reduce undesirable inhibitory outcomes [98]. Dual or multiple growth factor delivery regimens complicate the design of controlled release devices requiring separated compartments to provide sequential release. Such a system has been prepared that consisted of alginate-PLGA scaffolds with admixed VEGF and pre-encapsulated PDGF [99]. The two growth factors were then released at distinct kinetics and thereby produced a superior angiogenic effect as compared to VEGF or PDGF delivered separately.

7.4. Targeting approaches

Selective targeting of bone has mainly been addressed for systemically administered small molecular drugs and proteins. Nevertheless, also localized delivery might benefit from active targeting to specific bone sites or bone cells to improve efficacy and safety. Bisposphonates [100], tetracyclines [101], glutamic and aspartic oligopeptides [102], and peptides derived from non-collagenous proteins [103] have been used to deliver drugs to bone because of their calcium-binding properties. These bone-targeting moieties can be conjugated to polymeric backbones, for example to polyethyleneglycol or poly(*N*-2-hydroxypropylmethacrylamide) [104] to provide targeted delivery of growth factors. However, bone-targeting with Ca-binding moieties is rather unspecific and lacks interactions with specific cells. Surface modifications of biomedical devices loaded with therapeutic proteins, peptides, antibodies or DNA can be used to incorporate biomolecular recognition sites into drug delivery systems [105]. For example, a new class of copolymers based on poly(L-lysine)-*g*-poly(ethylene glycol) (PLL-*g*-PEG) has been used to functionalize microparticles or titanium implant surfaces with PEG-tethered integrin-binding adhesion peptides to target phagocytes [106] or to improve cell attachment, respectively [107]. Although not yet suggested for this purpose in the literature, the PLL-*g*-PEG system may be discussed as a potential platform technology that may be adapted for bone repair purposes, namely by conjugation of bone cell-specific ligands to the PEG side chain. Examples of potential PEG-tethered ligands could be osteoprogenitor-specific antibodies, e.g. HOP-26 and STRO-1 [108,109], calcitonin receptor inhibitors to target osteoclasts [110] or antibodies for alkaline phosphatase and parathyroid hormone-related protein receptor as osteoblast markers [111]. Such ligand-mediated interaction with the target cells would enable to direct the growth factor to be released more or less exclusively in close association with the target cells, possibly making growth factor delivery more specific and efficient.

8. Concluding remarks

The therapeutic success of growth factors will intimately depend on their optimal localized delivery in a given context. Modular delivery systems may have to be conceived that can be composed to match individual pathological situations. In the field of bone repair, one will have to account for the type of bone and its microarchitecture, the age and mobility of the patient, the size of the defect, and the natural cascade of events occurring during bone repair processes. Thus, the delivery system should not only release the best growth factor(s) at the right dose and kinetics, but further offer a matrix for the ingrowth of osteoprogenitor cells and blood vessels. It should provide mechanical congruency of the damaged bone from the start. Considering all this, one becomes aware that growth factor delivery for safe and efficacious therapy is still in its very early infancy. Only when the involved research groups succeed to bring together the best of relevant expertise from molecular biology, medicine, materials and pharmaceutical sciences, beneficial growth factor therapies will broadly emerge for the benefit of patients suffering from severely injured tissues.

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