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Factors stimulating bone formation

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Abstract The aim of this review is to describe major approaches for stimulating bone healing and to review other factors affecting bone healing. Spinal bone fusion after surgery is a demanding process requiring optimal conditions for clinical success. Bone formation and healing can be enhanced through various methods. Experimental studies have revealed an array of stimulative measures. These include biochemical stimulation by use of hormones and growth factors, physical stimulation through mechanical and electromagnetic measures, and bone grafting by use of bone tissue or bone substitutes. Newer biological techniques such as stem cell transplantation and gene therapy can also be used to stimulate bone healing. Apart from bone transplantation, clinical experience with the many stimulation modalities is limited. Possible areas for clinical use of these novel methods are discussed.

Keywords Bone healing · Bone graft · Growth factors · Stimulation · Stem cells · Gene therapy

Introduction

Osseous spinal fusion remains a cornerstone of surgical treatment of severe spinal disorders. However, the success rate is still debated and difficult to assess in the presence of metal implant material. A general failure rate of 5–30% is reported in lumbar spinal fusion [58]. Fusion capacity is influenced by numerous factors: individual biological factors, the bone graft material, and biomechanical and external factors. Predictive factors for good osseous fusion are uniformly associated with good functional outcome, for which psychosocial factors, the presence of neurodeficit, and the primary diagnosis also seem to have major importance.

The individual biological factors governing spinal fusion are related to bone homeostasis and thereby to age. Little is known about individual variations in spinal fusion capacity, although it is generally accepted that young patients heal well. Recent studies have shown that all osteotropic growth factors known to be sequentially involved in the spinal fusion process [6] are present in iliac crest bone autografts of all age groups but with higher variation in older people [3, 16]. Other growth factors that regulate vascular ingrowth have not been addressed and might be equally important. Osteotropic growth factors such as TGF β, BMP2, and BMP7 have been shown experimentally to induce/provide sufficient bone formation for both intracorporal and posterolateral spinal fusion at the level of bone autografts in sheep, rabbits, and baboons [9, 22], but the clinical effectiveness has yet to be determined in controlled studies and negative and positive results have been reported [7, 38, 39]. At present, the cost effectiveness of osteotropic growth factors for humans seems very high compared to combined femoral ring allograft and autograft for anterior interbody fusion or to morselized human allograft in posterolateral fusion.

Bone autograft harvesting is associated with high donor site morbidity (15–20%), even in long-term follow-up. Limited amounts of material are available in osteoporotic patients and for multilevel procedures. Bone cement, structural bone allograft, and metal spacers are viable alterna-

tives to bone autograft in anterior reconstruction. In many less invasive procedures such as cervical and lumbar fusion with cages, bone harvest is time-consuming, and alternative solutions with biologically active substances have found increasing popularity in these procedures [44].

The development of so-called biological cages for intercorporal fusion seems near. The primary goal is sufficient peripheral biomechanical stability. Then an osteogenic potential to bridge bone from one endplate to the other is required for long-term stability. Whether osteogenic growth factors are able to produce bone fusion alone or in combination with new carriers inside stress-shielding cages in older humans is an important question. In posterolateral fusion, additional support of the fusion process by supplementary growth factors or other osteoinductive principles would improve fusion and clinical outcome. However, the clinical effect of exogenic growth factors in the metabolic active fusion bed in humans is mostly unknown and needs further research.

The aim of this review is to present the current knowledge of stimulated bone healing and the basic biological mechanisms involved.

Principles for enhancing bone healing and bone formation

Three basic principles apply to the enhancement of bone healing and bone formation (Table 1). When investigating experimental enhancement, the following principles must be considered:

- Bone induction is new bone formation from determined osteogenic precursor cells
- Bone conduction is enhanced bone formation due to a favorable structural environment where bone is formed
- Bone genesis is stimulated by modulations of natural biochemical processes that initiate and maintain bone formation during a healing response

Bone induction can be obtained by two approaches: cell-mediated and growth factor-mediated. In the first, bone precursor cells can be harvested from bone marrow and placed in, for example, bone defects requiring bone induction to heal sufficiently. Bone precursor cells can also be cultured from crude bone marrow, and by this method large numbers of bone precursor cells can be obtained for bone induction. During osteoinduction, osteogenic precursor cells can proliferate in both osseous and nonosseous biological environments and differentiate to form mature osteoblasts, forming bone matrix that subsequently mineralizes to mature bone tissue.

Table 1 Basic principles for enhancement of bone healing and bone formation and their mechanisms

Osteoinduction	Osteogenesis	Osteoconduction
Bone precursor cells	Growth factors	Porous coatings
TGF-β superfamily	Ca/P ceramics	Ca/P ceramics
Bone autograft	Bone autograft	Bone allograft

In growth factor-mediated bone induction, a special family of growth factors called bone morphogenetic proteins (BMP) have the unique property of stimulating mesenchymal stem cells to differentiate towards chondro- and osteoblastic lineage. Local application of BMP induces extensive bone induction and can be used for bone formation in bone defects and as a substitute for bone grafts. After implantation of autologous bone graft, bone formation is probably accomplished by both cell- and growth factor-mediated bone induction, which occurs with viable precursor cells from the bone marrow stroma and BMP growth factors released from the bone matrix.

In bone conduction, normal bone formation is helped to extend due to a favorable structural environment in which the bone conductive material serves as a scaffold for new bone formation. An example of such an environment is a porous coating with the optimal pore size of 100–400 μm, which is able to favor bone ingrowth into endoprosthetic components. Allogenic bone grafts and other processed bone graft materials probably function mainly as bone conductive materials. Materials such as calcium phosphate ceramics and glass ceramics have osteoconductive properties but have also been suggested to stimulate osteogenesis by releasing nonorganic mineral ions, which activates cellular processes during bone formation and healing [62].

Bone matrix is a storage medium for growth factors that participate in activation and maintenance of cellular processes during bone formation and healing. Some of these growth factors have been shown to accelerate bone formation and bone healing when applied locally to intact and healing bone tissue. However, this principle is pure osteogenesis stimulation. The main topic of this review is to describe principles for stimulated bone healing and formation.

Patient-related factors

Age

Age is a definite determining factor for bone healing. One probable cause for reduced healing potential with increasing age is the reduction in the number of mesenchymal stem cells in bone tissue with increasing age [12]. With bone trauma, this means that stem cell recruitment for the healing response is reduced with increasing age. This limited access to biochemical factors during a healing response contributes to the already weakened healing response in older patients. Some evidence exists that the growth factor levels within bone matrix also decline with age [54, 59].

Osteoporosis

Osteoporosis leads to a decrease in bone volume, especially in areas with trabecular bone tissue. The reduced thickness and number of trabeculae resulting from osteoporosis compromise mechanical properties, especially of metaphyseal bone regions, and lead to increased fracture risk. Until recently, osteoporotic bone was thought to have the same healing potential as nonosteoporotic bone. However, recent studies demonstrated reduced fracture healing in osteoporotic rats [67].

Smoking

Smoking has been demonstrated to inhibit fracture healing and spinal fusion both experimentally and clinically [6, 36]. In experimental studies, nicotine demonstrated negative effects on callus formation, cortical remodeling, and mechanical properties [55]. In a spinal fusion model in rabbits, nicotine stimulation resulted in nonunion in all cases, as opposed to only 44% in the control group [60]. The negative influence of nicotine on the healing process is thought to be due mainly to inhibition of vascularization [56].

Drugs

Systemically administered drugs can potentially modulate bone formation and repair. Several drugs are known that have adverse effects on bone formation. Few anabolic hormones have been demonstrated to stimulate bone healing. Growth hormone has been demonstrated experimentally to enhance fracture healing [5]. No clinical study has demonstrated advantageous effects of growth hormone on fracture healing.

Biphosphonates, which block osteoclastic bone resorption, can increase bone mineral content (BMC) in osteoporotic patients. Some speculation existed as to whether interference with the bone remodeling process affects bone healing. Experimental and clinical studies have tested the effects of biphosphonates during fracture healing. In a randomized controlled study of patients with Colles' fractures, alendronate increased BMC at the fracture site and had no other adverse effects [1]. Similar effects have been found experimentally in rat diaphyseal fractures, with increased BMC and no effect on remodeling and mechanical properties of the fracture [46]. Today, no knowledge exists concerning the effects of bisphosphonates on the healing of bone grafts during spinal fusion.

Cytotoxic drugs used for chemotherapy can naturally exhibit negative effects on bone formation. Experimental studies have shown that even short-term administration of methotrexate reduces trabecular bone volume and the bone formation rate. Fractures in rats also heal more slowly and with weaker mechanical properties after methotrexate treatment [27, 53]. Corticosteroids have well-known adverse effects on bone remodeling, leading to osteoporosis. Also, bone healing is adversely affected by corticosteroids [25]. Nonsteroidal anti-inflammatory drugs (NSAID) are known to inhibit ectopic bone formation [29]. Some experimental data provide evidence that these drugs can inhibit fracture healing [28, 35]. Unfortunately, there is no certainty concerning the effects of NSAID on bone graft healing during spinal fusion or for osseointegration of spinal implants.

Adjuvant factors stimulating bone formation

Bone grafting

Principles

Bone grafts have two main functions: (1) promote bone formation or osteogenesis and (2) provide structural support. Osteogenesis may originate from the bone graft itself or from the host bed. Graft osteogenesis can occur when cells are transplanted, remain viable through diffusion, and produce new bone at the transplantation site. More significant contributions to osteogenesis, however, occur through the processes of osteoinduction and osteoconduction.

Osteoinduction occurs when pleuripotential mesenchymal cells in the host bed are recruited to differentiate into bone-forming cells known as osteoblasts. This process is mediated by BMP, a glycoprotein found in the matrix of bone grafts, and other growth factors [66]. Osteoconduction is the process whereby a bone graft serves as a scaffold or lattice facilitating migration of host cells for osteogenesis. Osteoconduction eventually leads to partial resorption of the graft and replacement by new host bone. This process is known as creeping substitution. Incorporation of bone grafts through osteoconduction, osteoinduction, and osteogenesis occurs in sequential phases similar to those of fracture healing. The length of time required for a bone graft to incorporate depends on a number of factors including the type of graft utilized and its structure.

Types of bone grafts

Three main types of bone grafts are used in clinical practice: autografts, allografts, and bone graft substitutes.

Autografts. Autogenous bone grafts (ABG) have been proven as the most reliable method of stimulating bone healing. Sources for these grafts include but are not limited to the patient's iliac crest, proximal femur or tibia, femoral head, and a resected rib. ABG incorporate more quickly than allografts by their greater capacity for osteoconduction and osteoinduction. Another advantage is that they may retain the capability of graft osteogenesis through

viable osteoblasts in the graft. Autograft application is the gold standard for obtaining spinal fusion during all kinds of spinal surgery.

Allografts. Because of the aforementioned disadvantages, alternatives to ABG have been researched extensively. Allografting (transplanting bone from one patient to another) has become a popular substitute. Allografts have mainly osteoconductive properties. Allograft chips are often used to supplement bone autograft when large amounts of bone graft material are needed during spine fusion.

Bone graft substitutes. To avoid immunologic graft rejection, eliminate disease risk, and have an unlimited supply of graft material, interest in bone graft substitutes has mounted. Bone graft substitutes include ceramics, demineralized bone matrix (DBM), and composite grafts. Most ceramics currently under investigation are synthetic and composed of hydroxyapatite (HA), tricalcium phosphate (TCP), or combinations of the two [15, 31, 48]. Ceramics simply provide an osteoconductive lattice upon which host osteogenesis can occur. They completely lack osteoinductive properties. Experimental animal studies have consistently demonstrated the superior performance of ABG over ceramic implants alone. However, Bucholz [15] found a similar efficacy of calcium phosphate ceramics and autogenous bone for certain clinical applications in humans such as filling defects under tibial plateau fractures, where the graft material would be under compressive forces. DBM is formed through acid extraction of bone, leaving a composite of noncollagenous proteins, bone growth factors, and collagen in continuity. Currently, DBM is available freeze-dried and processed from cortical or corticocancellous bone as a powder, crushed granules, or chips. It is also available as a gel called Grafton (Osteotech, Shrewsbury, N.J., USA), which can be applied intraoperatively with a syringe [52].

Composite grafts incorporate the favorable properties of different materials into a single compound. They include materials providing both an osteoconductive matrix and osteoinductive properties. Composites of TCP and BMP are currently in use, with TCP ceramic providing an osteoconductive matrix and the BMP stimulating bone healing through osteoinduction. Collagraft (Zimmer, Warsaw, Ind., USA) is a commercially available composite consisting of bovine fibrillar collagen and porous calcium phosphate ceramic. The mixture is nonosteoinductive, but adding autogenous bone marrow provides osteoinductive potential [18, 23].

Biochemical stimulation

Biochemical stimulation of bone healing is a new approach to the above mentioned clinical problems and has become increasingly relevant with the discovery more than a decade ago of peptide regulator molecules called growth factors. Growth factors have been found in all tissues and are today known to regulate local cell-to-cell metabolism and mediate the cellular effects of various hormones. Bone matrix is known to be a large reservoir for numerous growth factors that have been suggested as regulators of bone remodeling and initiators of the bone healing process [47]. In vitro studies have documented that bone growth factors exert numerous regulating effects on bone cells [73], while in vivo studies have shown that a small number of growth factors can stimulate bone healing processes in animals [4, 34, 71]. These data are promising for future possibilities in growth factor-stimulated bone formation and bone healing in orthopedic surgery.

Growth factors

Aside from structural proteins, bone matrix also contains small amounts of very potent regulators of bone cell metabolism. These proteins, called bone-derived growth factors, are produced by osteoblasts and incorporated into the extracellular matrix during bone formation, but small amounts can also be trapped systemically from serum and incorporated into the matrix. The growth factors are located within the matrix until remodeling or trauma causes solubilization and release of the proteins [17, 33]. After release, the growth factors are able to regulate osteoblast and osteoclast metabolism during bone remodeling and may initiate and control a healing response after bone trauma. Thus, they are recognized as the main regulators of bone cell metabolism.

Bone morphogenetic proteins

In 1965, Marshall Urist made the discovery that DBM could induce bone formation when placed ectopically in subcutaneous tissue [63]. This ability of DBM was ascribed to a protein which Urist named BMP [64, 65]. These are the only growth factors with a known ability to stimulate differentiation of mesenchymal stem cells in chondro- and osteoblastic directions [19, 64, 70]. Recent studies have demonstrated that BMPs are expressed during the early phases of fracture healing [32, 50]. The novel recombinant BMPs have intact bone-inducing capacity but need special carriers to exert their activity at low doses [24, 45, 57, 68]. Functional carriers for BMP are: collagen matrix, DBM, and various synthetic polysaccharide matrices [72].

The function of the carrier matrix is to immobilize bone-inducing protein at a particular site for a sufficient time to allow bone induction to occur. In vivo studies of BMP have primarily focused on its use in stimulating the healing of bone defects [21, 61, 71]. A novel clinical approach was performed by Cook, who used BMP-7 and

collagen as a substitute for autologous bone in spine fusions in dogs [22]. Although the cellular mechanisms for BMP-stimulated bone induction are only vaguely understood, the in vivo bone induction activity of this group of growth factors is unique, and BMP appears very promising for clinical use in any situation requiring stimulation of bone healing.

Transforming growth factor β

Transforming growth factor beta (TGF-β) is a multifunctional cytokine with a broad range of biological activities. These include regulation of growth and differentiation of many cell types. In general, TGF-β has stimulative effects on cells of mesenchymal origin. Applied continuously to a healing osteotomy in rabbits, TGF-β stimulated increased callus formation and increased maximal bending strength of the osteotomy [43]. In vivo studies have demonstrated stimulatory effects of recombinant human (rh)TGF-β1 on bone healing to both unloaded and weight-loaded TCP and HA ceramic-coated implants in dogs [40, 41, 42]. The in vivo data on TGF-β's ability to stimulate bone formation are very promising, and TGF-β and BMP are probably the most realistic candidates as growth factors for stimulating bone healing and bone induction in orthopedic surgical situations.

Clinical experience with growth factors

So far, only limited data exist concerning the effects of growth factors on human bone tissue and in clinical scenarios. Growth factors in the BMP family are currently being tested and under evaluation for clinical use. BMP-2 and BMP-7 (the latter also designated osteogenic protein, or OP-1) have been tested mainly in clinical cases for salvage purposes. One randomized clinical trial was performed using OP-1 in combination with type 1 bovine collagen. The study addressed treatment of pseudarthrosis. Autogenous bone graft was tested against OP-1 in a randomized multicenter study including 150 patients [20]. Patients were followed for 1 year. Results of the study demonstrated equal effects of OP-1 and autograft treatments according to patient-based clinical evaluation. Radiographic evaluation demonstrated a slightly higher degree of healing in the autograft group. This result, however, could be biased because the autograft used has an independent radiodensity, whereas the OP-1 does not. One controlled study of the effects of OP-1 on bone formation in humans was performed by Geesink [26]. Patients with tibial osteotomies for surgical treatment of osteoarthrosis had additional fibular defects that were stimulated with OP-1 in combination with type 1 bovine collagen, collagen alone, empty gap, and DBM. The OP-1 stimulated healing of the defects, whereas collagen alone did not.

In conclusion, research during the last decade has shown increasing evidence that growth factors can be used as in vivo stimulators of bone healing and bone formation and therefore have therapeutic potential in a variety of clinical situations in orthopedic surgery.

Cell-mediated bone healing

Recently, efforts have focused on the possibility of osteogenic cell transplants for enhancing bone healing and formation. Osteogenic stem cells have been identified, characterized, and isolated for culture expansion [30]. These cells, which are retrieved from bone marrow, can be grown and multiplied in vitro. Multiplied osteogenic stem cell suspensions can be used as transplants for local implantation at sites requiring enhanced bone formation. Preclinical studies have demonstrated the in vivo potential for bone regeneration of locally implanted osteogenic stem cells. Healing of critically sized defects in long bone has been demonstrated in rats and dogs by the use of culture-expanded autologous osteogenic stem cells combined with porous ceramic implants [13, 14]. Bone formation and mechanical properties were enhanced in groups whose ceramic implants were loaded with osteogenic cells. Combinations of osteogenic stem cells and growth factors have been shown to stimulate bone healing in critically large defects. A combination of BMP-2 and nonpurified marrow cells demonstrated 100% healing at 6 weeks, whereas BMP-2 alone, marrow cells alone, and autologous bone graft all demonstrated less than 100% healing at 12 weeks [37]. Cell-mediated stimulated bone healing is very interesting clinically because of the easy access to bone marrow and the high degree of safety using autologous tissue. Additional preclinical studies and clinical studies are necessary to define indications and effects of this new treatment modality.

Gene therapy of bone healing

Osteogenic stem cell transplantation has been further developed using principles of gene therapy. Gene therapy for local enhancement of bone healing is accomplished by transfecting bone stem cells with genes encoding for growth factors that stimulate bone healing. The stem cells then function as growth factor factories with overexpression of a single growth factor. Other phenotypic characteristics of the stem cells which favor bone formation are retained. Typically, the growth factor overexpression is maintained for several weeks. Stem cells transfected with growth factors of the BMP family have been the most successful. Recent studies have described successful stimulation of bone formation using adenovirus-mediated direct gene transfer of BMP [2, 49]. In these studies, direct injections of BMP expressing adenovirus or cells transfected

with BMP virus were able to induce bone formation in muscle tissue. More clinically related animal studies were performed in a long bone defect model and a spine fusion model. In the former, marrow cells were transfected with BMP-2-expressing adenovirus [39]. The BMP-2-expressing marrow cells were subsequently injected into critically sized defects in rat femur. A substantially higher healing rate was seen in the BMP-2 marrow-treated group than with BMP-2 protein alone or marrow cells alone. In the spine fusion study, DBM was soaked with marrow transfected and subsequently expressing a novel osteoinductive protein called latent membrane protein (LMP)-1 [8]. Fusion rates were 100% in the LMP marrow cell group and 0% in the marrow cell alone group.

The principle of gene therapy is very interesting as a future clinical tool for stimulation of bone formation and healing, and extensive research is being performed to determine potential advantages and indications for this method. However, concern exists about clinical safety issues and the long-term complications of injecting genetically altered cells into humans.

Physical factors

Electrical

Electrical stimulation of bone healing has existed for three decades and is an FDA-approved treatment for nonunion. The theoretical and biological background for why electrical stimuli can accelerate bone repair is not fully understood, but some knowledge is available. Electrical processes are thought to play a significant role in the mechanically mediated stimulation of bone healing and bone modeling. Charged molecules of bone matrix proteins can generate streaming potentials during mechanical deformation. Also, ion flux can be generated in interstitial fluid of bone tissue during mechanical deformation [69]. Such endogenous electromechanical phenomena affect osteoblasts and osteocytes and alter transmembrane ion channel properties, leading to increased metabolic activity.

Three different types of electrical stimulation have been developed for clinical use. Direct current generators use 5–20 mA. The anode is placed cutaneously some distance from the stimulation site [11]. Alternating current generators use a 5 V, 60 kHz signal to develop a 5–10 mA current at skin level. These methods are also called capacitively coupled electrical stimulation. Using a noninvasive approach, electrodes are placed on each side of the extremity being treated. Pulsed electromagnetic fields (PEMF), also called inductive coupling, represent a different concept in which electrical currents are induced magnetically to microenvironments such as cells and extracellular matrix [10, 51]. Presently, electrical stimulation has obtained limited clinical application. For fresh fractures, this effect is so moderate and therefore clinically insignificant. For nonunion, more recent treatment methods combining intramedullary nailing and bone grafting are successful in up to 90% of cases. This has diminished the relative advantages of electrical stimulation for treating nonunion.

Conclusion

Numerous stimulatory modalities for bone healing have proven effective in preclinical experimental settings. However, limited clinical data exist that address what types of stimulation are beneficial to patients. Also, no data exist concerning which pathological conditions respond to stimulation of bone healing. The growth factors from the TGF superfamily, BMP and TGF, have demonstrated potent stimulatory capability for bone healing and bone formation in vivo. The major problem of these growth factors in clinical use is appropriate delivery systems to ensure sufficient biological activity for optimal effects on bone healing and formation. In the future, the use of bone grafting could probably be reduced considerably by employing growth factors in collagenous or other matrices. Animal experiments have demonstrated that BMP can form bone to the same extent as autologous bone for spine fusions in dogs [22]. New techniques such gene therapy and autologous bone stem cell transplantation might help improve stimulation approaches so that bone grafting can be reduced.

The biological state of the bone tissue to be stimulated is important for the possibility of obtaining stimulatory effects. Most studies on stimulation of bone healing were performed in healthy bone with good vascularization. It should be speculated that impaired vascularization and poor bone quality could reduce the stimulatory effects of growth factors and other adjuvant therapies. However, it could also be speculated that exogenous stimulation is especially advantageous when endogenous healing is compromised. Additional studies are needed to answer these questions and to find ways of improving the biological response to stimulation with various qualities of bone and vascularization.

One major problem is to identify patients and conditions in which impaired bone healing response is likely to lead to poor clinical results such as nonfusion after spinal stabilizing procedures. Therefore, new diagnostic tools are needed that test preoperative healing capacity. This would enable surgeons to determine which patients need adjuvant therapies to improve bone healing.

The coming decade will possibly define indications for the new clinical tools for stimulating bone healing in clinical practice. These tools could increase fusion rates after spine surgery and reduce morbidity after autograft harvest.

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