Born Again Bone: Tissue Engineering for Bone Repair

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Destruction of bone tissue due to disease and inefficient bone healing after traumatic injury may be addressed by tissue engineering techniques. Growth factor, cytokine protein, and gene therapies will be developed, which, in conjunction with suitable carriers, will regenerate missing bone or help in cases of defective healing.

During the Paleozoic period, evolution produced the skeleton. This 500 million-year-old structure, like the liver and central nervous system, has the capacity for regeneration, a term until recently restricted to new tissue formation seen in hydra, planarians, and salamanders. Why are we thinking about the regeneration of bone? The stimulation of bone production is often required to treat loss of bone tissue brought about by trauma, osteonecrosis, and tumors. Currently, bone-grafting procedures are employed to promote healing of fracture nonunion (in which the ends of the bone do not heal together), in craniofacial reconstruction procedures, and in fusion techniques for spinal surgery. The clinical gold standard for bone repair is an autologous graft (grafts from the individuals themselves), which, although effective, is limited by the availability of sufficient donor tissue and problems with donor site morbidity. These limitations have led to the development of both biological agents to induce bone growth and substitute tissue grafts using different cell types, attached to a variety of matrices or scaffolds.

Treating nonhealing fractures

There are ~6.5 million fractures per year in the United States, of which ~15% are difficult to heal. For those fractures in which the healing is slow (delayed union) or does not occur (nonunion), there are few effective therapies at present. The most common method of treatment is insertion of bone from the individual (autologous) or from alternate sources (autogenous) into the defect. In this procedure, bone is removed from a variety of sources, most commonly the pelvis following a surgical incision made in the hip area. The donor bone tissue is inserted at the nonhealing fracture site, and additional support may be provided by an orthopedic rod or plate. More than 250,000 bone grafts are performed annually in the United States in an attempt to assist the body in regenerating new bone lost by trauma or disease. Poor fracture healing is associated with chronic pain and prolonged ambulatory impairment and must often be treated by surgical intervention. This has considerable economic implications for healthcare providers. External fixation devices may stabilize fractures at risk from poor healing, although a lack of viable bone at the fracture site may result, at best, in the production of unstable bone that is prone to refracture. Although bone grafting is generally successful, it suffers from the limited amount of donor tissue that may be available, and the patient may suffer side effects such as numbness or tingling at the donor site, infection, or prolonged pain. An alternative therapy involves the use of pulsed electromagnetic fields, which have been shown to have effects on many aspects of bone formation and healing. This includes the induction of endothelial and bone cell proliferation, bone formation of capillary sprouts, the stimulation of matrix formation, and calcification. A further recent addition is the use of low-frequency ultrasound. The role of physical forces in stimulating bone and tissue repair in general will be discussed in a later section.

What happens when a bone is fractured?

A fracture is a break in a bone that is always associated with damage to the surrounding tissues. The amount of damage is related to the energy that created the break. The fracture is called “open” when the skin envelope around the bone is breached and “closed” when it remains intact. Soon after a fracture occurs, a number of events proceed to initiate the healing and repair process. First, growth and differentiating factors are activated by the injury process, which in turn stimulate localized pluripotent osteoprogenitor cells. These produce a class of proteins known as bone morphogenetic proteins (BMPs), which are intimately bound to collagen. BMPs belong to the transforming growth factor (TGF)-β superfamily of peptide growth factors (8). The key BMPs found to have bone-forming activity are listed in Table 1. These osteoinductive proteins, along with other growth factors, cytokines, and hormones, induce the migration of mesenchymal cells and their proliferation and differentiation into bone-forming cells. As in the skin and other tissues, bone repair is a continuous process that sets a cascade of events into motion. These events are shown in Figs. 1 and 2. The healing of bone includes an initial rapid inflammatory response (minutes to hours), chemotaxis and mitosis (hours to days), production of extracellular matrix, remodeling of the injury site, and localized angiogenesis (days to weeks). Bone remodeling is ultimately brought about by primary intramembranous bone formation, chondrogenesis, and endochondral ossification, with production of bone matrix leading to reconstitution of bone continuity. In addition to the BMPs, a number of other growth factors, listed...
in Table 2, are produced after injury that may, in their own right, serve to stimulate osteogenesis and repair bone. These factors will be discussed in a later section, are not specific to bone, and are an integral part of the platform that supports tissue repair in both hard and soft tissues.

**Tissue repair: common mechanisms in injury processes?**

When a bone is fractured, skin is torn, blood vessels are ruptured, or ligaments or muscles are damaged, the tissue acquires a “wounded phenotype.” Tissue repair results from a number of temporally coordinated processes driven by locally released mediators. The first event is immediate and consists of the activation of the coagulation cascade and the formation of a blood clot. Shortly afterward there follows an acute inflammatory response resulting in tissue edema and cytokine and growth factor release. Then follows the first stage of collagen repair, involving deposition and the formation of granulation tissue, which becomes a new and temporary weak tissue. The third and final process is the second phase of collagen repair, resulting in extracellular matrix remodeling, angiogenesis, and the reproduction of full-strength tissue. Much of the normal healing process is driven by growth factors and cytokines (20). In addition to their role in bone clot formation, platelets and mesenchymal cells generate a number of growth factors that are found in wound fluid, including TGF-α, platelet-derived growth factor (PDGF), epidermal growth factor, vascular endothelial growth factor, TGF-β, and insulin-like growth factor-1 (IGF-I). In this acute inflammatory response, neutrophil migration is induced by PDGF, interleukin-1α and -8, tumor necrosis factor (TNF)-α, granulocyte macrophage-colony stimulating factor, and granulocyte-colony stimulating factor.

Numerous growth factors have been implicated in the repair of fracture healing, which include acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), PDGF, and TGF-β. Thus multiple growth factors and cytokines play a major role in tissue repair. The BMPs that are produced by osteoblasts and mesenchymal cells merely represent one level of tissue specificity among a plethora of ubiquitous stimulatory proteins. Thus the wounded phenotype “tags” injured tissue separately from uninjured healthy tissue. This may provide a potential therapeutic route to enhancing the healing process or slowing down the proliferative response in diseases such as diabetic retinopathy and dermal scarring in soft tissue and production of extraneous bone in patients with fibrodysplasia ossificans progressiva (FOP).

**Mechanical forces and bone repair**

The concept that bone formation and remodeling is controlled by both physical and biochemical factors was first proposed over 100 years ago. It has been suggested that bone contains sensor cells, possibly osteocytes, that monitor mechanical stress and activate adaptive biological processes to remodel the bone. Osteocytes, which are distributed throughout the bone, produce a signal through electrical potential activation of ion channels, flow of interstitial fluid, and activation of signaling cascades that is in direct proportion to the amount of mechanical loading (15). The initial mechanotransduction event in cells contained in bone is very similar to that found in endothelial cells that line the cardiovascular system. In both cases, transactivation of target genes involves stimulation of mitogen-activated protein kinase signaling cascades, as well as protein phosphorylation and dephosphorylation, and ultimately results in the direct binding of transcription factors to gene promoters. The mechanical shear stress that is the stimulus in both systems causes transcriptional activation via distinct transcription factor binding sites that function as shear stress response elements (4). These elements are found in many promoters such as PDGF-B, TGF-β, and FGF (13) that are activated by injury. Transcription factors themselves, such as early growth response factor-1 (Egr-1) and stimulating protein-1 (Sp-1) are also responsive to mechanical stimuli such as shear stress (18). At a fracture site, it appears that an optimum level of mechanical stress is a route to fast and effective healing, for example, distraction osteogenesis is a surgical procedure that may be used to correct limb length discrepancies or deformities or to treat nonunion fracture following acute loss of functional bone, for instance after a crush injury. In this procedure, an osteotomy is performed followed by the application of an external fixator device such as an Ilizarov frame. After a period of ~1 wk, distraction is performed at controlled rate and the bone begins to grow and will eventually fill the gap, growing at a rate of ~1 cm/1~2 mo distraction time. Recent studies in a rabbit model of distraction osteogenesis have shown that the expression of BMP-2, -4, and -7 is increased after distraction is
initiated and maintained throughout the distraction period (17). In addition, in vitro studies in rat osteoblasts exposed to pulsed electromagnetic fields showed that expression of BMP-2 and -4 was increased, which was accompanied by an increase in mineralized bone nodule formation (1). These observations are consistent with physical forces inducing BMP expression and subsequent stimulation of new bone formation. Finally, it has been known for some time that cartilage regeneration may be enhanced by drill hole injury into subchondral bone. One possible explanation for this regenerative effect may be mechanotransduction, in which injury-induced stimulation of immediate-early genes (IEGs) drives the production of growth-stimulating proteins. BMP-4, for example, stimulates the production of the IEG Egr-1, which may feed back to stimulate BMP-4 expression and which in turn serves to promote tissue repair by activation of multiple growth factors and cytokines (see below).

Agents to induce bone repair: preclinical studies

There are many agents known to induce bone formation in vitro and in animal models of ectopic bone formation or acute injury, for example osteotomy. Some of these factors are aFGF, bFGF, TGF-β, PDGF, IGF-I, and BMP-2, -4, and -7 [osteogenic protein (OP)-1], Indian and Sonic hedgehog, and parathyroid hormone. TGF-β has received considerable attention in the promotion of both soft and hard tissue repair. TGF-β has been shown to stimulate the three-dimensional cellular development of human bone ex vivo, a process that is prerequisite for successful tissue-engineered bone (12). Observed effects with TGF-β in vivo have, however, been variable, and one study reports no beneficial effect of this factor in a rabbit model of distraction osteogenesis. Of this collection of growth factors, BMPs have proven to be the most potent stimulators of osteogenesis, and recombinant human (rh) BMP protein has been used to heal intermediate-sized bone defects in a variety of animal models, including rats, dogs, rabbits, and nonhuman primates. It should be borne in mind, however, that an animal model of acute bone injury may not necessarily reflect the inability of bone to heal, such as in nonunion fracture, periodontal bone regeneration, spinal fusion, or revision total joint surgery. One such example relates to the use of BMP-3 (osteogenin), which in humans failed to promote periodontal regeneration despite showing powerful osteoinductive capacity in animal models (14).

Agents to induce bone repair: clinical studies

To date there have been a number of clinical studies using BMPs to promote bone repair by using either rhBMP-2 or rhOP-1 (BMP-7) protein on a collagen scaffold (3, 6). Although some promising results have been obtained, there appears to be a varying response from individual to individual in terms of new bone production. rhBMP-2 protein was used to augment maxillary sinus and alveolar ridge formation and to form new bone in tooth extraction pockets. rhBMP-2 has been used in a small number of patients with hip osteonecrosis; the protein was implanted at the site of core decompression, a process that removes the dead bone from inside the femoral head. BMP protein allografting has also been shown to be effective in the reconstruction of femoral nonunion in patients with failed fracture healing. OP-1 has been used to treat patients undergoing

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**FIGURE 1.** Biochemical responses to bone fracture. Bone fracture and tissue damage activate many biochemical processes. Some occur within minutes, such as an acute inflammatory response, activation of immediate-early genes, and stimulation of signaling cascades, e.g., mitogen-activated protein kinase signaling. Some occur within hours, such as release of growth factors and cytokines. Then, within days, development of repair blastema and angiogenesis occur, which enables full tissue repair within weeks.
maxillary sinus floor elevation and unstable thoracolumbar spinal fractures as well as in a randomized study of bone healing after fibula osteotomy. Although deemed to have a successful outcome, large amounts (in some case milligram quantities) of BMP were used in some studies. This may not be commercially viable and could also have an undesirable side effect profile. Such potential limitations may be obviated by the use of a better carrier or delivery system, for example, by the use of polymer matrices, an adeno-virus expressing the BMP-2 gene, or an autologous cell therapy in which disaggregated cells are propagated in vitro to a synthetic scaffold and implanted at the site of injury. However, the patient-to-patient variation in response to administration of BMP protein may, in part, be due to the induction of natural BMP repressor proteins, such as noggin, follistatin, and chordin. This suggests that a more subtle modulation of the natural response to tissue healing, in which a cascade of multiple growth-inducing factors is stimulated, may result in a gradual and sustained production of new bone.

In addition to the therapeutic use of the BMPs, several growth factors have been shown to induce bone formation in the clinic. In studies of 38 patients with moderate-to-severe periodontal disease aimed at periodontal and peri-implant bone regeneration, the safety and efficacy of a combination of rhPDGF-BB and rhIGF-I was evaluated. Patients who received PDGF/IGF-I responded with 43.5% osseous defect fill, whereas the control group showed 18.5% osseous defect fill (10).

Transcription factor gene therapy to promote tissue repair

When bone is injured, one of the earliest events to occur is the activation of intracellular signaling cascades. These cascades serve to stimulate the rapid transcription of growth factors and cytokines, and they are initiated, at least in part, by the stimulation of IEGs. Egr-1 (also called NGFI-A, Krox24, Zif268, and TIS8) is an IEG coding for an 80-kDa phosphoprotein transcription factor. Egr-1 belongs to the C2H2 class of zinc finger proteins that includes Egr-2 (also called Krox-20), Egr-3, and Egr-4 (also called NGFI-C). Egr-1 is naturally produced in response to acute stimuli, and the Egr-1 promoter comprises transcription factor binding sites that allow modulation of Egr-1 transcription by a number of physiological stimuli. These stimuli include hypoxia, laminar fluid shear stress generated in vitro or in vivo, mechanical stretch, bone loading, and acute tissue injury. Egr-1 transcription may also be induced by nonphysiological stimuli such as electric shock, phorbol ester, radiation, and urea. The induction of Egr-1 protein stimulates the production of many genes whose products play a role in cellular growth, development, and differentiation. These include genes encoding cytokines (TNF-α), adhesion molecules (intercellular adhesion molecule-1), members of the coagulation cascade (tissue factor, urokinase-type plasminogen activator), and growth factors such as aFGF, bFGF, TGF-β, PDGF-A and -B, hepatocyte growth factor, vascular endothelial growth factor, and IGF-II. Gene gun delivery of Egr-1 DNA
has been shown to promote healing following dermal wound-
ing in healthy rodents, in which it was shown to promote
angiogenesis in vitro and in vivo, enhance reepithelialization,
increase collagen production, and accelerate wound closure
(5). In addition, Egr-1 is capable of inducing bone formation in
a rodent model of ectopic osteogenesis. These studies demon-
strate that Egr-1 can accelerate the normal healing process and
raise the potential use of this pleiotropic transcription factor as
a therapy for any aspect of tissue repair.

Recent studies have shown that another transcription factor,
Fos-related antigen-I (Fra-I), may also increase bone formation
(11). Fra-I is a c-Fos-related protein belonging to the activator
protein-1 (AP-1) family of transcription factors. In transgenic
mice overexpressing Fra-I, there was an increase in the num-
ber of mature osteoblasts and a progressive increase in bone
mass of the entire skeleton. This study suggests that Fra-I stim-
ulates bone formation by promoting osteoblast differentiation
and may represent a further pathway for therapeutic interven-
tion to increase bone mass after acute injury or during later life in
patients with osteoporosis.

Delivery of osteogenic substances

So far we have described a number of potential protein or
gene therapies that may be used to induce bone repair. How-
ever, both the safety and efficacy of biological therapies will be
considerably enhanced by the use of an efficient delivery vehi-
cle or matrix. Vehicles for gene delivery are numerous and
include viral constructs such as retrovirus, adenovirus, ade-
noassociated virus vectors, and nonviral delivery approaches
such as cationic lipids and liposomal formulations. Matrices
for gene or protein delivery that provide a stable and sustained
release of the agent have been the subject of research for many
years and are now beginning to show promise. Some studies
have used allogenic cortical bone, whereas others have inves-
tigated the use of synthetic substances. These matrices or scaf-
dolds are biocomposites of either natural or synthetic macro-
molecules such as those constructed from bone substitutes,
copolymers, or bovine collagen (2) and represent the corner-
stone of the first generation of biodegradable implants. Other
means of inducing both soft and hard tissue repair involve the
use of genetically engineered pluripotent mesenchymal cells
(7) or marrow stromal cells (MSC), a subset of stem-like cells
that can now be extended almost without limit in vitro without
losing their potential for differentiation. MSC when mixed with
coral implants have been shown to stimulate bone regenera-
tion and achieve clinical union in a animal model of bone
defect (16). A preliminary clinical trial shows promise for the
treatment of osteogenesis imperfecta, a bone disease charac-
terized by a mutation in the type I collagen gene (9). In this
study, allogenic bone marrow transplantation was shown to
lead to engraftment of functional mesenchymal progenitor
cells (MSC) that, three months after osteoblast engraftment,
stimulated total body bone mineral content.

Limiting the repair process

The ability of osteoinductive proteins to stimulate bone heal-
ing offers considerable promise to patients who present with
recalcitrant bone repair. However, what limits bone produc-
tion? In patients with the rare autosomal dominant disease
FOP, bone formation occurs in the muscle, tendons, ligaments,
and other surrounding connective tissue, resulting in partial or
complete immobility. Patients with FOP have constitutively
increased expression of BMP-4, which may explain their
limitation of motion. BMP-4 expression is downregulated by the
natural antagonists follistatin, chordin, and noggin. The use of
noggin gene therapy to counteract unwanted bone formation
in patients with FOP is being explored. Thus it may be neces-
sary to carefully control the level of BMP-4 expression such
that its activity is sufficient to promote local bone formation
but is insufficient to promote undesirable bone formation.
It is also paramount that our natural repressor proteins retain
the ability to act as a “brake” on this potent osteoinductive
cytokine.
In summary, the next few years will bring together exciting and rapidly developing areas of biomedical research, which will involve the collaborative disciplines of bioengineers, molecular biologists, physiologists, and drug and gene delivery specialists. The constant discovery of new genes and the exploitation of gene targets with previously characterized functions will help spur on the evolving science of regenerative medicine.

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References