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The Future of Bone Healing

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Bone healing has emerged as a dynamic field of research whereby science attempts to augment nature by enhancing and expediting the course of fracture union. With the advent of possibilities from bone substitutes, growth factors, and stem cell research, the potential for enhancing bone healing is vast. This article attempts to survey current trends and to highlight upcoming techniques in the future of bone healing.

Necessity often drives science; therefore, the question arises as to whether there has been an increase in the occurrence of bone nonunion and delayed union. In the United States alone, surgeons perform an estimated 500,000 to 600,000 bone grafting procedures annually [1]. The rate of nonunion and delayed union has been quoted between 5% and 10% [2], and there are no definitive studies comparing these rates from decades ago.

We can make generalizations and observations about our population as a whole. In most developed countries, diabetes has increased to epidemic proportions. In 1995, epidemiologists estimated the prevalence of adult diabetes worldwide at 4% and projected it to rise to 5.4% by 2025; a significant increase in the prevalence in adults over age 65 years was also projected [3]. Wound and bone healing is problematic for people who have diabetes because these patients have greater susceptibility to infection and delays in healing. Infections are also more difficult to treat, particularly in light of an alarming trend toward drug-resistant bacteria occurring particularly in people who have diabetes and in those who are immunocompromised. Furthermore, medical treatment of patients who have diabetes becomes even more difficult because many are obese.

Increases of food portion sizes and consumption of high-caloric convenience foods exacerbate the obesity trend. Modern conveniences and transportation

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allow people to expend less energy, thereby intensifying the problem. In most developed countries, a more sedentary lifestyle has resulted in dramatic increases in childhood obesity rates. In the past 20 years, the prevalence of obesity has more than doubled in boys and girls. In addition, school budget cuts in North American public schools have jeopardized physical education classes and after-school sports programs [4].

Over the past century, despite the significant rise in diabetes and obesity, life expectancies have increased, thus leading to an increasing proportion of people in the elderly population. Issues with osteoporosis, delays in bone healing, and susceptibilities to infection can complicate fracture healing in the elderly. Compliance is difficult for the obese and elderly, especially when their fracture or surgery requires non–weight bearing status to heal. Being non–weight bearing or using crutches can be extremely difficult or impossible for some patients. With all these additional variables, present-day physicians are forced to treat a more difficult patient population base than physicians from decades ago.

The emergence of modern-day illnesses and trauma has led to the evolution of modern medicine. The advent of motor vehicles and sophisticated firearms forced surgeons to find the best treatment for high-velocity injuries, gunshot wounds, and associated open fractures. Fractures occurring in areas with marginal vascularity—arising in patients who have metabolic bone disease or in patients undergoing chemotherapy—also experience difficulty healing. Again, necessity drives science and medicine to fine-tune its modalities to make treatment more patient specific. Acting alone, any illness combined with a difficult fracture can be disastrous for a patient, but more often than not, a surgeon sees combinations of multiple comorbidities when treating a difficult fracture.

In the past 10 to 15 years, as surgical cases became increasingly complex, advances in bone healing have surfaced that have been critical in limb salvage. Distraction osteogenesis using the Ilizarov technique has been useful in cases involving severe bony comminution, tumor resection, congenital malformation, or osteomyelitis. Bone distraction allows the filling in of the deficit by a millimeter per day and, in some cases, allows early mobilization, which is particularly important in the noncompliant patient who cannot or will not tolerate non–weight bearing. Bone graft may not be appropriate in areas with huge deficits, especially with long medullary bone or in cases that need angular correction and lengthening. Spatial frames have evolved to correct angular deformities in long bones by gradual correction. Through mechanical stress of the bone after an osteotomy, external fixators can induce bone healing by pulling at the fracture site at a very well controlled rate. Precise preoperative planning is essential, and includes good full-length tibiofibular radiographs and the identification of the planes of deformity. At times, deformities are found in more than one bone or joint so that correction of one problem may unmask a varus or valgus component in another area. Thus, careful preoperative planning should consider more than one center of deformity. Measurements taken from the radiographs are entered into the spatial frame computer program that generates the appropriate settings for periodic adjustments of the frame struts. This surgery

permits the patient to regenerate his or her own bone over time, typically bypassing any need for bone graft.

External fixators can also be used to stabilize and or decompress severe fractures. Application of these fixators are particularly appropriate for fractures resulting from gunshot wounds or severely comminuted fractures that cannot hold fixation. Pilon fractures often involve impaction of the articular surface, resulting in limb shortening. External fixators can distract these fractures to attempt to restore length to the limb. When attempting to heal bone with overlying large wounds or fractures involving infection, external fixation may be ideal. The pins can be placed to stabilize the proximal and distal ends of the fracture yet avoid the problematic area of healing. This methodology allows the skin envelope to heal and the surgeon to debride the wound or infected bone. Mini external fixators are of particular use in compression or nutcracker fractures of the cuboid, in crush fractures of the navicular, or in comminuted fractures of the metatarsals. Internal fixation can be used in combination with external fixation; however, it is often the case that for crush or comminuted fractures, only the use of external fixation for stabilization of fracture fragments is necessary. Depending on the soft tissue envelope and the degree of impaction, external casting may not always be a viable option for stabilizing the fracture. By restoring the length of the fractured bones and providing stability, external fixators can allow the body to mend the fracture without the need for bone graft. They have proved to be versatile in treating complex deformities. Manufacturers of external fixators continually research ways to improve these frames by making them easier to use, stronger, lighter, and radiolucent.

Nevertheless, with more than 5.5 million fractures and 1 million bone repair surgeries annually, bone graft plays a significant role in aiding fracture repair. Delayed or nonunions constitute 10% to 15% of the annual fractures in the United States [5]. Synthetic bone grafts comprise approximately 10% of the bone graft market, and an estimated 500,000 to 600,000 bone grafting procedures are performed annually in the United States alone [1]. To attain ideal bone healing conditions, bone grafting material should exhibit three physiologic properties: osteogenesis, osteoinduction, and osteoconduction [6]. Osteogenesis refers to the formation of new bone from the living cells that are transplanted with the bone graft. Osteoinduction refers to the process whereby a multitude of growth factors and hormones within the extracellular matrix influence mesenchymal stem cells to be recruited and to migrate to the site of bone formation and subsequently differentiate into viable osteoblasts. Osteoconduction refers to the graft's ability to function as a structural lattice or scaffold for stability and for cells to infiltrate. These osteoconductive properties physically support the bony structure and allow ingrowth of capillaries, stem cells, and differentiated cells for the purpose of graft incorporation by the host. Cancellous bone offers a porous osteoconductive environment, whereas cortical bone offers structural integrity. The goal of bone grafting for fracture repair is to mimic corticocancellous autogenous bone by creating a good balance of osteogenesis, osteoinduction, and osteoconduction. Often, bone graft or bone graft substitutes lack

one of those components, which necessitates exogenous additions of osteoinductive products. Much current research focuses on these products, and this topic is discussed later in this article.

Traditionally, fresh autograft was considered the best bone graft for orthopedic procedures. Autografts were typically taken from the ipsilateral iliac crest, creating a second surgical site, which can be fraught with complications including nerve damage, bleeding, prolonged pain, and difficulty with postoperative ambulation. Harvesting grafts from a second surgical site typically prolongs hospital stays and significantly increases the cost of the procedure. Cadaveric allograft has shown significant merit, with numerous advantages over autograft, including supply, storage, and accessibility [1,6]. With the morbidity of a second surgical site and with more studies demonstrating the success and wide utility of allograft, surgeons are more inclined to avoid autograft when possible. All allogenic bone grafts used today come from cadaveric donors. Before implantation, all viable cellular components are destroyed during the processing of the allogenic bone graft, thus the risks of host rejection and viral disease transmission are minimal.

Various synthetic bone substitutes and bioceramics are available at this time. Most are composed of synthetic calcium hydroxyapatite, calcium sulfate, or calcium phosphate derivatives, which provide structural stability and the scaffold needed for bone healing. Calcium phosphate has been used for decades in bone surgery because it closely resembles human cancellous bone and is absorbed between 10 and 12 weeks [7]. Calcium sulfate hardens more than calcium phosphate and provides more structural strength. Hydroxyapatite provides minimal structural strength and is nonimmunogenic but degrades after 18 months post implantation. Other solid resorbable polymer scaffolds are composed of polylactic acid, polyglycolic acid, polyethylene, or polyprolylene [8]. These biomaterials should be biodegradable without eliciting a significant inflammatory response and should ensure adequate cell infiltration to be incorporated into existing bone. A study of several synthetic nonallograft bone graft substitutes was performed to compare composition, histology, and indications [1]. This study compared Food and Drug Administration (FDA)-approved synthetic bone graft substitutes by brand name and researched their indications and future development. Side-by-side comparison studies such as this are useful for the surgeon in a clinical setting and should be performed on all implantable materials. Newer constructs have been used that combine the osteoconductive scaffold with the osteoinductive mesenchymal cells. The biomaterials must ensure adequate cell integration without eliciting an inflammatory reaction. These synthetic bioceramic matrices serve as delivery vehicles for the mesenchymal cells while providing structural support for a large bony defect and fixation [9].

Tissue engineering is undergoing rapid evolution in podiatric and orthopedic surgery with the selection of cells, cytokines, and matrices. Allogenic bone graft alone is unable to regenerate bone without the signals of the cells in the grafted site. Most, if not all, of the role of bone graft is osteoconductive. The relationship between the graft or matrix and the osteoinductive progenitor cells is crucial

for the integration of new tissue. The scaffold provides the structural strength and stability while the osteoinductive agent promotes osteogenesis. More controlled clinical trials should be performed that compare the histology and indications of osteoinductive synthetic agents in conjunction with autografts, cadaveric allograft, and synthetic matrices. Each application should consider the ideal recipe of the osteoconductive and osteoinductive properties to create the best environment for bony regeneration. Clearly, creating the perfect protein chain by selecting the appropriate molecules and then the appropriate sequences is a daunting task. Is the protein created with the assumption that all bone healing in all patients is the same? It would seem logical to surmise that bone regenerative capacities differ significantly among individuals of the same species. A patient who has vascular compromise, diabetes, or nutritional deficiencies would have a different osteogenic potential than a healthy patient.

Bone marrow aspirates containing stromal cells are capable of differentiating into osteoblasts, chondrocytes, and adiopocytes. Depending on the microenvironment, mesenchymal cells differentiate into bone precursor osteogenic cells. Locally acting proteins drive the differentiation pathways of the mesenchymal cells. Bone formation and resorption are processes that are closely regulated by these local factors. Cells that participate in the process of fracture repair include platelets, fibroblasts, inflammatory cells, osteoclasts, and osteogenic precursors. Current investigations headed by biotechnology companies focus greatly on recombinant proteins in fracture repair. Bone morphogenic proteins, fibroblast growth factor, platelet-derived growth factor, and angiogenic factors such as vascular endothelial growth factor (VEGF) are among the most studied proteins today. Currently, one of the most popular techniques in enhancing bone healing during surgery involves the use of platelet concentrates to supplement the fracture sites (Symphony, Depuy Spine, Raynham, Massachusetts). During surgery, the patient's blood is centrifuged to separate the protein-rich platelets that presumably contain the elements essential to bone healing. This concentrate is applied directly to the osteotomy or fracture site to enhance bone healing.

Some of the more studied proteins shown to be osteoinductive and critical in bone healing are the bone morphogenic proteins (BMPs); in particular, BMP-2, BMP-4, BMP-7, and BMP-9 [10,11]. There are at least 20 BMPs that collectively fall under a larger category of proteins called transforming growth factor β. One of the most well researched BMPs is BMP-2. Research has shown that BMP-2 is successful in stimulating bone growth and regeneration in spinal fusions [12] and open tibial shaft fractures. BMP-2 binds to receptors on the mesenchymal cells and signals them to differentiate into cells that eventually become cartilage- or bone-forming cells [13]. BMP-2 acts only locally and appears to be upregulated in its production at areas of fracture. In one study, chondrocyte differentiation was shown to be greatest when BMP-2 is applied at the peak day of 32, and improved chondrogenesis is shown when BMP-2 acts in synergy with other supplements [14]. This same study also noted when certain differentiations led to increased adipogenesis. Finding the causes of increased adiopogenesis can improve osteogenesis. If the adipogenic amino

acid sequence can be inhibited, then this may cause upregulation of osteogenic cells and factors.

Another growth factor of particular interest is the angiogenic factor called VEGF. VEGF has been shown to stimulate bone repair by promoting angiogenesis. Currently, VEGF inhibitors are used in oncology therapy to stop angiogenesis and suffocate the tumor expansion. VEGF is required for normal fracture repair and enhances osteoblastic activity in vitro. VEGF is important in early angiogenesis, especially 7 days post fracture. VEGF has been shown to be involved in the conversion of soft cartilaginous callus to hard callus. It also stimulates chemotaxis of osteoclasts. The proteins that immediately promote osteogenesis and angiogenesis should be incorporated at the time of fracture repair. In the early stages of bone repair, the VEGF concentration is highest in the fracture hematoma [15]. Studies show that inhibition of VEGF also inhibits BMP-2. VEGF also appears to work in synergy with BMP-4. When combined, there were increases in mesenchymal stem cells, which accelerated the bone healing process. This enhancement of bone healing only occurs with the appropriate ratio of VEGF to BMP-4. Conversely, improper ratios of VEGF to BMP-4 can elicit detrimental effects [5]. Similar assumptions can be made about the synergistic effects of various other proteins. Although it seems intuitive, randomly adding all growth factors shown to be individually beneficial to bone healing to a fracture site may be detrimental. More research is needed to determine the ideal ratios of proteins required for optimized bone healing [16].

Various studies have shown that the local volume or concentration of a particular growth factor increases gene expression. Most of the growth factors appear to act locally at the fracture site. At particular concentrations, with delivery at particular times, there appears to be a several-fold increase of osteogenic or chondrogenic activity with various proteins. In one study, the degree of chondrogenic differentiation was increased 1.6-fold when 10 ng/mL versus 2 ng/mL of BMP-2 was added [14]. In animal studies of spinal fusions, posterior fusions could be achieved without decortication by using large doses of BMP [12]. Spine surgeons perform similar fusions by injecting BMP and allograft and bypassing the need to delaminate vertebrae.

The timing of the delivery for these proteins during the various stages of bone healing can also maximize the results. Some growth factors have little effect on the acute fracture, thus the protein may have to be given locally—not at the time of the fracture repair but perhaps during the second phase of bone healing. For example, BMP-2 is believed to play a role in later chondrogenesis and osteogenesis. This protein needs to be effectively delivered to the fracture site. Various biodegradable carriers, perhaps in the form of synthetic bone graft, have been researched in the delivery of osteoinductive proteins. Commercially available collagen sponges have been shown to facilitate the implantation of the protein and retain the protein at the site of implantation [13]. In particular, using fluoroscopic assistance during later stages of bone healing by injecting the protein directly into the healing fracture could maximize the effect of BMP-2. Continued

research on which proteins to use, the appropriate dosage, and the timing of application is crucial in engineering bone healing.

Mesenchymal cells found mostly in human bone marrow are capable of differentiating into osteogenic cells depending on local conditions of their microenvironment. During their time of development, proteins and other signals can influence the cell to differentiate into various specialized cell lineages such as osteogenic, chondrogenic, and adiopogenic cells. Much of recent research has focused on autologous bone marrow stromal cells and their interactions with growth factors. Future research should also focus on allogenic bone marrow stromal cells from healthy human donors. If the fracture patient has difficulty healing a fracture due to poor protoplasm, radiation therapy, or other problems, bone marrow cells of the same patient would, by the same reasoning, have limited differentiation potential.

Stimulation of mesenchymal cells alone may not be adequate in healing large bony defects. Gene transfer techniques are able to stimulate the mesenchymal cells to continuously express the osteogenic genes, resulting in sustained production of a particular protein such as BMP. Gene therapy strategies shown in animal models can deliver BMPs using the two major classes of vehicles for gene transfer: viral and nonviral vectors. Viruses are efficient vehicles for transferring genetic material into cells; however, as with any viral invasion, the host may mount an immune response and become resistant to gene transfer after continuous exposure. Current approaches include deleting the viral genes that are most immunogenic and preserving the genes desired in the transfer. There are two basic methods of gene transfer: ex vivo therapy, whereby cells are removed, genetically modified, and transplanted back into the same recipient; and in vivo therapy, whereby genetic materials are directly transferred into the patient. In the ex vivo technique, tissue can be harvested from the patient to infect the cells in vitro before loading the cells into a biodegradable carrier to be implanted in the patient's bone. The ex vivo gene therapy approach has potential advantages, including direct delivery of the genes to the desired area and the potential to manipulate gene expression in the laboratory under controlled conditions before implantation. With the in vivo technique, the vectors can be injected into the host to infect the mesenchymal cells [11,17–19].

There are conflicting opinions about which technique has more utility in bone healing. With the in vivo technique, the level of expression is high initially but declines to undetectable levels by the second week. With the ex vivo technique, the level of expression is lower initially but the duration can last up to 6 weeks. There is no inflammatory response with the ex vivo technique; however, it is a technically difficult and time-consuming process [20]. Ideally, the advantage of gene transfer is the continuous expression of the gene after the transfer. Other implantation methods not involving gene transfer only provide a bolus or pulse dose of the protein, which is temporary. After gene transfer, sustained expression of the gene provides continuous delivery of the osteoinductive proteins at the fracture site for a longer period than direct implantation [17,20,21].

Adjunctive fracture therapy has involved the use of bone stimulators, especially in the past 2 decades. Electrical stimulation was first found to stimulate osteogenesis. More recent studies agree that electrical stimuli improve angiogenesis critical to wound and bone healing [22]. Low-energy electrical currents applied to bone function in normal bone remodeling and repair. Oscillating electromagnetic fields have proved effective in generating electrochemical gradients across cell membranes [23]. Several types of bone stimulators exist, from implantable to external applications. These bone stimulators typically function with parallel coiled capacitors generating a pulsed electromagnetic field or with electrodes attached to a battery pack. The implantable bone stimulators have a higher degree of success because the electrodes are placed directly in the fusion site and generate direct current, ensuring 100% patient compliance. The cathode is fairly sizeable and additional surgical exposure is necessary. A second minor surgery is typically required to remove the bone stimulator after the fusion.

Similar applications of autograft, allograft, and gene transfer techniques apply to cartilage repair in joints. For cartilaginous defects such as nonhealing talar dome lesions, most of the approaches for repair involve transplantation of differentiated autologous chondrocyte cells obtained from biopsies of similar cartilaginous tissue. With this mosaicplasty technique, there is a limited availability of cells and morbidity at the donor site. Experimental studies are being performed to transplant mesenchymal precursor or stem cells. Obtaining stem cells from bone marrow, for example, has less surgical morbidity, and these cells presumably have the potential to differentiate into cartilaginous or bone cells [8].

Another area of upcoming research for bone healing involves bone xenografts. Porcine- and bovine-derived tendon and vascular grafts, intestinal mucosal substitutes, and animal-derived wound coverings are commonly used today in general surgery, and the question naturally arises as to whether bone xenograft is possible in the future. Similar to autologous bone graft, xenograft needs pasteurization to decrease chances for any immunogenic host reaction but is safe from any humanly transmitted diseases such as HIV and hepatitis. Immunosuppressive drug therapy may be required during the incorporation of the graft, but several studies have shown that animal-to-human disease transmission has not occurred [18]. Graft rejection poses the most likely complication; however, recent international cases of Creutzfeldt-Jakob disease, the avian flu, and severe acute respiratory syndrome have caused concern over cross-species disease transmission. As with any implant, safety is the most important concern, and more research is needed to determine the efficacy and safety of xenografts.

Systemic supplementation of calcitrol, phosphorus, calcium, and vitamin D has been researched in persons who have rickets and other metabolic bone disorders [24,25]. The only bone anabolic hormone known to increase bone mineral density is human parathyroid hormone (PTH) 1 to 34. PTH has been tested successfully in animals and humans and has recently been approved by the FDA for treatment of patients who have advanced osteoporosis [26]. PTH may

have potential application in fracture repair; however, other anabolic hormones for bone healing are being researched. Bisphosphonate therapy has been used in treatment of Charcot's syndrome and osteoporosis, but more definitive benefits in treatment need to be researched. Appropriate nutritional supplementation (including zinc and calcium) and its benefits in aiding bone healing also need further research.

Safety, efficacy, ease of utility, supply, and cost effectiveness are all factors to be considered when engineering these bone healing supplements. Recruiting recombinant proteins such as BMP and other factors to heal bone appear to be the future; however, this process is costly because large amounts of these proteins seem to be required to stimulate bone repair and these proteins only act for a short duration of time. Gene transfer techniques appear to bypass this problem because they create sustained expression of the proteins.

The cost of developing and researching any tissue-engineered device involving biologically active agents is substantial, with estimates of $200 to $300 million, and require at least 8 years of research and development to complete the FDA approval process [11,27–34]. Developing osteoconductive acellular substances is a comparably faster process; however, estimates of cost still vary from $5 to $200 million.

Undoubtedly, bone healing research has immense financial cost and potential revenue for biotechnology companies. These costs will transfer to insurance companies and patients, thereby raising health care costs by some immeasurable margin. Clearly, costs are considered of minimal consequence if these products facilitate or augment bone healing in complicated patients who would otherwise receive longer than average immobilization or face possible amputation. Gene transfer therapy is a dynamic topic that is wide open for research. Future research will simplify the process of gene therapy, perhaps making it more efficient and less laborious. Most likely, various bone healing techniques and products will be used in synergy to maximize patient benefit. Discovering new techniques for bone healing is only part of what is required to advance this field of research. The surgeon's role is just as essential in this process. The surgeon has the responsibility to learn the latest techniques to ensure that the patient has the greatest chance of a successful result. He or she should first identify a high-risk patient and then tailor the treatment to the patient's specific needs.

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