Skeletal Repair in Distraction Osteogenesis: Mechanisms and Enhancements

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» Distraction osteogenesis, utilized for reconstruction of skeletal deformities and bone defects, encompasses three phases of repair that are distinct from those of fracture-healing: latency, distraction, and consolidation. During distraction, osteogenic potential is maintained because of a number of molecular, cellular, and mechanical influences.

» Many protein signaling pathways contribute to skeletal repair during the different phases of distraction osteogenesis. During distraction, bone morphogenetic proteins (BMPs) and their signal transduction molecules (Smads) influence osteoblasts to induce continuous bone formation. Transforming growth factor-beta (TGF-β) may be important in suppressing mineralization during distraction.

» Mechanical tension, controlled by the rate and rhythm of distraction, influences cell proliferation, angiogenesis, and genetic expression in the distraction gap.

» Multiple animal models and small human trials have demonstrated the beneficial effects of systemic and local adjuncts to distraction osteogenesis.

» Despite recent translational and clinical advancements, the application of osteogenic enhancements during distraction osteogenesis must be considered carefully. High-speed distraction may result in painful neuropathy and soft-tissue complications.

Distraction osteogenesis is a surgical procedure for the reconstruction of skeletal deformities associated with fracture malunion, congenital deformities and developmental conditions, bone defects, and limb-length discrepancies (Fig. 1A)[1]. In distraction osteogenesis, an osteotomy is performed and is followed by gradual distraction to utilize mechanical strain to induce the integration of cells, growth factors, and extracellular matrix to form bone. In most cases, distraction osteogenesis creates an environment that suppresses the formation of cartilage and encourages angiogenesis with subsequent intramembranous bone formation; in some cases, instability results in callus and partial endochondral bone formation[2].

This process undergoes phases that are distinct from fracture-healing. The three phases of distraction osteogenesis are

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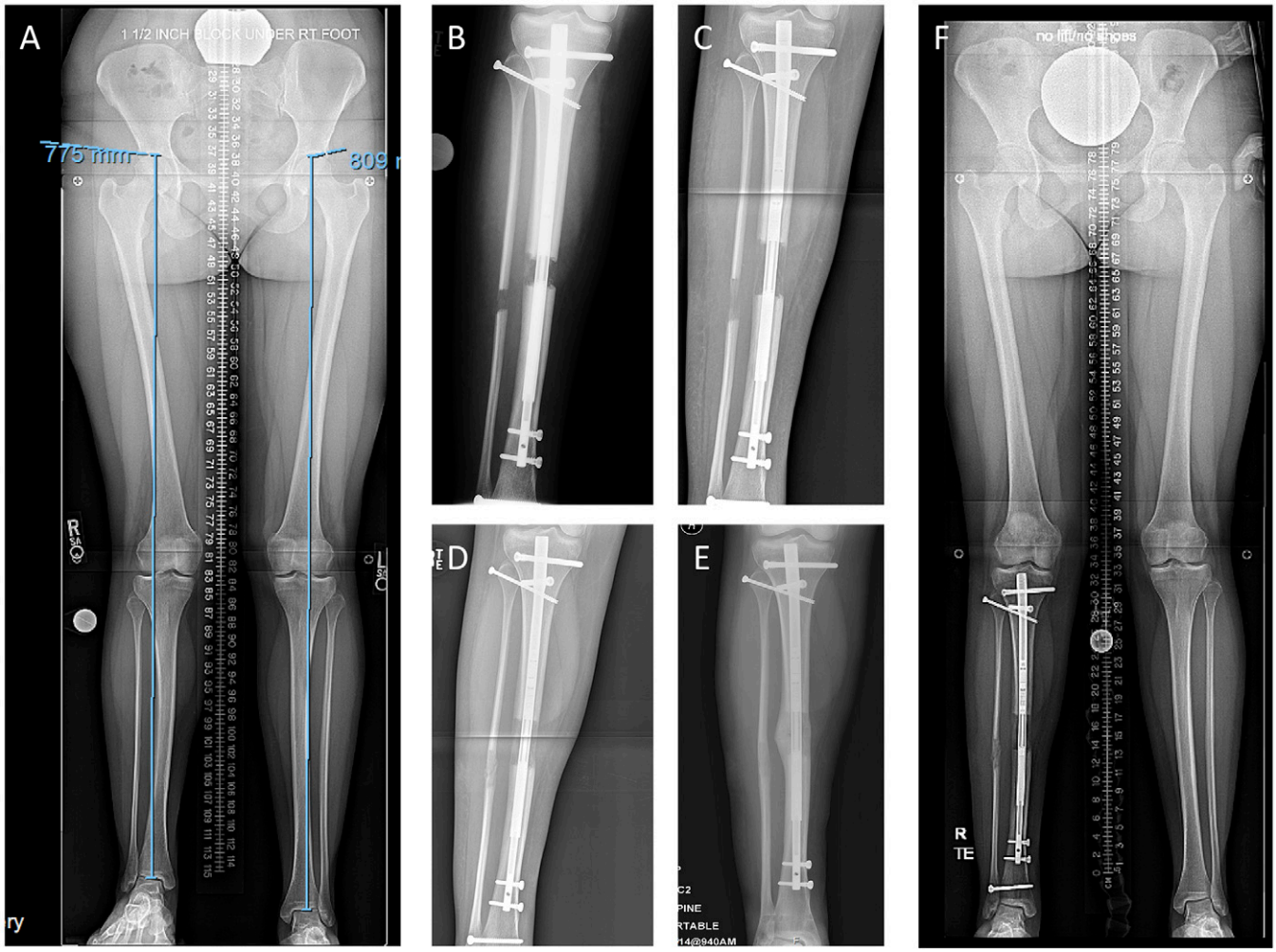

Fig. 1

Radiographic presentation of each phase of distraction. **Fig. 1A** Preoperative imaging demonstrating a limb-length discrepancy due to right tibial shortening. **Fig. 1B** Early distraction. **Fig. 1C** Late distraction. **Fig. 1D** Early consolidation. **Fig. 1E** Late consolidation, with evident osseous union. **Fig. 1F** Full-length radiograph demonstrating that the limb-length discrepancy has been corrected.

latency, distraction, and consolidation[3,4]. In latency, the primary inflammatory response occurs immediately following surgical osteotomy, a process that is similar after fracture. The period of time allowed for latency is clinically determined on the basis of the patient's healing potential and may last from three to ten days (e.g., pediatric patients require less time in latency)[5-7]. In distraction, the callus is subjected to mechanical forces, forming a fibrous interzone[1,8,9] characterized by active chondrocyte-like cells, osteoblasts, and fibroblasts. On radiographic examination during distraction, callus formation can be detected three to six weeks after distraction initiation (Fig. 1B and Fig. 1C)[10]. Of note, the duration of the latency phase and the rate and rhythm during the distraction phase can influence the balance between nonunion and premature consolidation. Attentive clinical and radiographic follow-up is crucial to achieving the desired correction. Finally, in the consolidation phase (Fig. 1D), mineralization and remodeling occur, resulting in osseous union of the distraction gap (Fig. 1E and Fig. 1F). The time required for complete consolidation is variable: the pediatric population may only require one month per centimeter lengthened, whereas adult populations may require 1.5 to two months of consolidation per centimeter lengthened[6]. Although the initial healing after osteotomy is most closely related to fracture-healing, great efforts have been expended to elucidate the osteogenic responses throughout the distraction period and the basic science that dictates variable healing potential throughout the three phases[11].

Multiple methods of fixation and distraction are available for distraction osteogenesis, utilizing either internal or external fixation. Externally, unilateral external fixation or a circular external fixator is routinely employed (Fig. 2). The rings of the circular fixator are attached to proximal and distal bone segments with half-pins and wires. Each ring is also attached to adjacent rings with threaded rods or struts, forming hexapodal strut-linked platforms. These

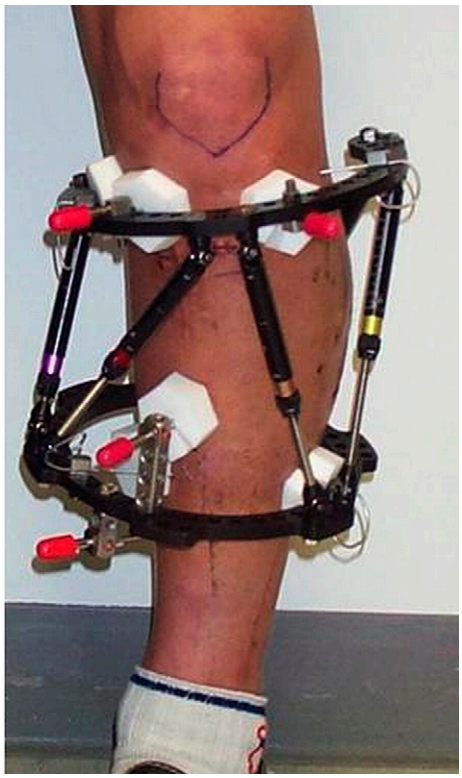

Fig. 2
A classic circular external fixator with rings affixed to bone with pins and connected externally with struts.

threaded rods are gradually adjusted to the desired length and position of the bone segments. Internally, surgeons may choose an intramedullary lengthening nail (Fig. 1). In lengthening over a nail, an older integrated fixation technique, an intramedullary solid nail is placed at the time of osteotomy, and an external frame is used to distract the bone segments.

In addition to device selection, multiple clinical decisions influence healing during distraction osteogenesis. Surgical osteotomy can be performed in a variety of ways; however, preservation of the periosteum and blood supply is critical for bone formation[12,13]. Depending on the site and type of implant used, a Gigli saw, osteotome, oscillating saw, or multiple drill-hole technique can be utilized for the osteotomy. Evidence from a dog model suggests that the multiple drill-hole technique results in improved healing compared with an oscillating saw osteotomy[14]; however, in patients undergoing tibial lengthening, the Gigli saw technique showed a significantly improved healing index ($p = 0.022$), suggesting a biologically superior technique[15]. Additionally, the selection of osteotomy site may be indicated by a variety of clinical factors, including the deformity, anatomy, clinical strategy, and biological considerations such as the condition of the soft tissues[16]. For example, it is crucial to avoid performing osteotomy of unhealthy bone of little regenerative potential. In children, the femur heals faster than the tibia, likely because of the surrounding musculature and vascularization[17]. The metaphysis has better bone-healing potential, likely because of more vascularity and a larger osseous surface, compared with the diaphysis[16]. However, the metaphysis is also a site of multiple muscular insertions and thus requires higher distraction loads[1]. The appropriate osteotomy site and techniques are determined by weighing multiple clinical factors.

Despite the best clinical practice, optimal clinical outcomes are not always possible. The aim of this review is to describe the complex molecular, cellular, and mechanical mechanisms in distraction osteogenesis and recent research efforts to employ natural mechanisms to augment bone regenerate formation.

Molecular and Cellular Mechanisms of Distraction Osteogenesis

The molecular and cellular activities during distraction osteogenesis are distinct from normal bone-healing and underscore the profound adaptability of skeletal healing. Although the latency phase closely resembles early fracture-healing within the osteotomy site, the expression of an array of bone-active proteins upon the initiation of distraction alters the local environment of the distraction gap and further impacts the mechanisms of consolidation and remodeling. The expression of these proteins is tightly temporally controlled throughout each phase of distraction osteogenesis (Fig. 3).

In the latency phase, formation of a soft callus closely mimics initial bone-healing seen in fracture repair. For example, the trauma of surgical osteotomy increases cytokines interleukin-1 (IL-1)

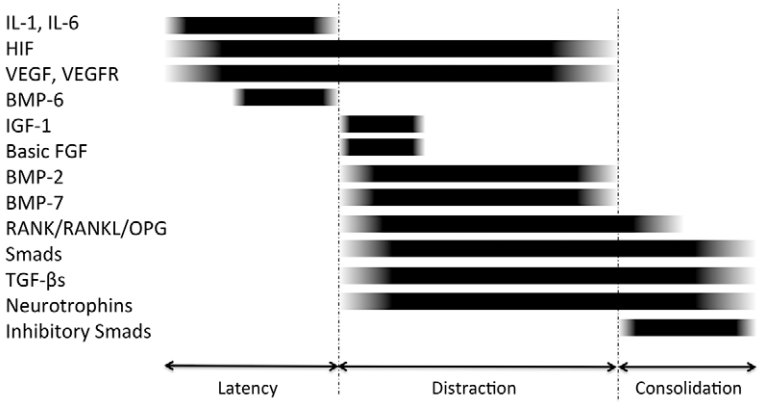

Fig. 3
Molecular signaling in distraction osteogenesis. Temporal genetic expression of various molecules in distraction osteogenesis influences maintenance of osteogenic potential. Latency, distraction, and consolidation constitute unique molecular environments.

and IL-6. A study of rat tibial distraction osteogenesis showed that IL-6, specifically, is expressed beyond the initial latency phase of healing and into the distraction phase by cells in the fibrous interzone, including osteoblasts and chondrocytes[18]. IL-6 responds to tensile strain in the distraction gap, inhibiting the differentiation of mesenchymal cells into mature osteoblastic lineage cells, suggesting that it is key in delaying maturation of the callus[11].

Angiogenesis is crucial to successful regeneration of the skeleton[19,20]. Under hypoxic conditions, as are commonly found at sites of trauma or decreased vascularity, the expression of hypoxia-inducible factor (HIF) activates angiogenic factor expression[21], thus increasing oxygen supply at sites of skeletal trauma. Evidence suggests that a hypoxic environment supports an osteocytic phenotype, whereas osteoblastic differentiation and bone formation depend on normoxic conditions[22]. HIF mediates oxygenation by recruitment of vessel formation and thus directly influences bone cell differentiation by mediating oxygen tension and nutrient availability. The presence of all major vascular endothelial growth factor (VEGF) ligands and interactive molecules, such as neuropilin and placental growth factor, has been detected in both fracture-healing and distraction osteogenesis, underscoring their importance in normal bone regeneration[23-26]. Evidence suggests that VEGF receptors (VEGFRs) 1 and 2 are essential for both the formation of new blood vessels and new bone formation through skeletal cell differentiation. A partial blockade of the VEGF pathway is selective for chondrogenesis, whereas a complete blockade results in the failure of osteogenesis and chondrogenesis[27].

BMPs are multifunctional growth factors implicated in bone formation[28]. BMPs are differentially expressed during each phase of distraction osteogenesis[29-32]. BMP-6 is strongly expressed in late latency and diminishes during distraction[29]. Transient expression of BMP-4 is observed in the latency phase[31-33]; however, in a study of rat distraction osteogenesis, BMP-2 and BMP-4 were strongly expressed in chondrocytes and osteoblasts and their precursors throughout distraction[29], contributing to uninterrupted bone formation. After the cessation of distraction, expression of BMP-2 and BMP-4 gradually resolves[30,34].

Smad proteins are involved in transducing BMP signaling intracellularly[35,36]. In a rabbit model of tibial distraction, Smad protein expression was negligible during the latency phase; however, Smads were maximally expressed in chondrocytes and fibroblasts during distraction and consolidation[37,38]. Receptor-activated and common-partner Smads (transducing molecules in the BMP pathway) were strongly expressed during distraction, although expression of inhibitory Smads (antagonists of the BMP pathway) was increased during consolidation, thus inhibiting BMP signaling[37-39]. The expression of BMP-2, BMP-4, and Smad proteins is consistent with bone deposition during distraction followed by gradual tapering in the consolidation phase as the callus cellular processes transition from mineralization to remodeling. Other growth factors, such as insulin-like growth factor 1 (IGF-1) and basic fibroblast growth factor (basic FGF), are implicated in osteoblastic precursor cell recruitment; they are induced at distraction initiation and return to basal levels during consolidation[40-42].

Transforming growth factor-beta (TGF-β) is a family of proteins that have complex effects on cells of mesenchymal origin[43,44]. In mandibular distraction osteogenesis in a rat model, the TGF-β1 expression increased at the onset of distraction and remained elevated for four weeks after the completion of distraction and onset of the consolidation phase[45]. Although TGF-β1 has been found to stimulate osteoblast proliferation, high levels suppress osteocalcin expression and induce osteoclastogenesis and thus may delay mineralization in distraction osteogenesis[46-48].

Neurotrophins promote differentiation and survival of neuronal cells[49]. In an experimental rat femoral distraction osteogenesis model, neurotrophin expression during the distraction osteogenesis exceeded the levels found in fracture models[50,51]. Peak neurotrophin levels occurred during distraction and tapered rapidly at the commencement of consolidation[37]. Expression of neurotrophin-3 by osteoblast-like cells and subsequent tropomyosin-receptor kinase (Trk) receptor expression may

suggest an autocrine loop function in distraction osteogenesis[52].

The receptor activator of NF (nuclear factor)-kappa β (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) system is essential for homeostasis of the skeleton, regulating resorption and remodeling activities of bone cells[53]. Control of cell differentiation, proliferation, and apoptosis is paramount for osseous tissue remodeling and repair[54]. Osteoclast-inhibitory OPG messenger RNA (mRNA) peaks during distraction and remains increased for up to two weeks of consolidation[55]; during a similar period, there is increased tissue inhibition by metalloproteinase 1, an extracellular matrix turnover regulator, that ultimately favors bone deposition[41]. RANKL, an osteoclastogenic cytokine, steadily increases during the consolidation period and remains highly expressed until three or four weeks of consolidation, thus indicating remodeling activity within the distraction gap[56].

Initial inflammation, cytokine expression, and cellular recruitment are key in the latency phase. However, distraction relies on a complex interaction of multiple cascades to form malleable bone regenerate in the distraction gap. Bone remodeling during consolidation is inhibited until distraction is complete as dictated by cellular reaction to changes within the distraction gap.

Basic Mechanical Mechanisms of Distraction Osteogenesis

The mechanical strain applied to the distraction gap influences cellular activities and gene expression, ultimately altering healing and osteogenesis. Mechanical tension is applied by choosing an appropriate distraction rate and rhythm for each patient. It is integral to maintaining a balance between nonunion and premature callus formation.

Mechanical tension during distraction osteogenesis affects cellular synthetic processes as well as differentiation of pluripotent cells in the distraction gap[57,58]. In vitro, osteoblasts subjected to cyclic stretching both stimulated proliferation and increased cellular production of additional mitogens such as TGF-β[47]. The mechanical tension in the distraction gap is directly related to the rate of distraction. The rate of distraction must balance bone regeneration, muscle development, and angiogenesis. Slower rates of 0.3 to 0.7 mm/day are best for muscle generation and angiogenesis, as well as type-I collagen production[47,59,60], whereas a rate of 1.0 mm/day is most favorable for osteogenesis[61]. The rate of distraction influences the distribution of collagen within the callus; as the distraction rate increases, the number of cells expressing type-II collagen mRNA increases, correlating with the generation of chondrofibrous tissue in the distraction gap[9,61]. The implication is that the slowest rate of distraction that produces regenerate tissue without premature consolidation should be used during distraction osteogenesis.

Weight-bearing during distraction osteogenesis may alter overall mechanical strain. A study on rat femoral distraction osteogenesis showed stimulation of blood vessel formation in physiologic weight-bearing compared with non-weight-bearing[62]. Mechanical strain may be a determinant factor for chondrogenesis and inhibition of osteoblastic lineage[63,64]. Distraction increases production of other extracellular matrix proteins as well, including osteonectin, osteopontin, and osteocalcin.

Local and Systemic Adjuncts in Distraction Osteogenesis

Augmentation of bone formation has been explored as a beneficial adjunct to distraction osteogenesis (Table I). One of the most devastating complications is fracture of the regenerate after external fixation removal[65]. Adjuncts to promote callus formation have been administered either systemically or locally at the osteotomy site. Acceleration of osseous healing may, in turn, increase limb lengthening potential and may decrease the time that patients must wear a bulky external fixator[66]. In a new era of bone lengthening with a mechanical intramedullary nail, enhancement of bone-healing to shorten the consolidation phase would allow patients to return to full weight-bearing more quickly and would decrease the likelihood of implant failure[67].

Intravenous infusion of alendronate during distraction osteogenesis in rabbits has shown promising results in increasing peak bone mineral content around the lengthened segment[68]. Initially, bisphosphonates were used in the prevention of external fixator-related osteoporosis[69,70]; in these studies, the distraction gap was found to be shorter than in control groups, perhaps because of premature consolidation.

Bisphosphonates have successfully rescued insufficiency of bone formation in patients undergoing distraction osteogenesis[71]. In a rabbit tibial model of distraction osteogenesis with and without continuous high-dose alendronate infusion, the volumetric bone mineral density, cortical bone thickness, and mechanical strength of the treatment group were substantially improved compared with control animals[68]. Although long-term use of bisphosphonates forms relatively higher-quality callus, mature bone formation and remodeling are delayed because of osteoclast inactivity[72,73].

In a similar experiment on rabbit tibiae, continuous infusion of calcitonin was compared with alendronate infusion, and it was found that all histologic parameters were not significantly different; however, the torsional failure load was significantly improved in the calcitonin treatment group ($p = 0.006$)[74].

Systemic administration of nerve growth factor (NGF) may also address other complications of distraction osteogenesis, such as sensory disturbances and peripheral neuropathy[75]. In a fracture model, NGF has been shown to stimulate bone formation around regenerating axons[76], and it improves fracture-healing in rats[77]. Locally, NGF mRNA expression is increased during distraction and early consolidation[78], and Wang et al. showed that local application of NGF causes acceleration of fracture callus maturation at the onset of consolidation[79].

TABLE I Summary of Systemic and Local Adjuncts in Distraction Osteogenesis

Reference	Subject	Treatment	Result
Systemic			
Kiely et al.[71]	Human	Bisphosphonate	Rescue from bone formation insufficiency
Abbaspour et al.[68]	Rat tibia	Continuous high-dose alendronate	Improvement of bone regenerate quality
Sen et al.[74]	Rat tibia	Calcitonin compared with alendronate infusion	Calcitonin significantly improved torsional failure load
Du et al.[75]	Rabbit mandible	Nerve growth factor	Acceleration of recovery of transected inferior alveolar nerve
Local			
Yonezawa et al.[80], Cheung et al.[155], Mizumoto et al.[83], Cheung and Zheng[156], Li et al.[84], Mandu-Hrit et al.[82], Haidar et al.[85]	Rat and rabbit	BMP-2, BMP-7	Promoted bone regeneration at normal and high-speed distractions
Zhu et al.[88]	Rabbit tibia	BMP-2 and NELL-1	Enhancement of osseous healing compared with BMP-2 alone
Kroczek et al.[86]	Goettigen mini-pig mandible	BMP-2, BMP-7, IGF-1, TGF-β	BMP-2 and BMP-7 were significantly more osteogenic
Ali et al.[92]	Rabbit tibia	Platelet-rich plasma	Enhanced consolidation
Moore et al.[96]	Rat femur	PDGF	Enhanced bone-healing
Fujio et al.[108]	Mouse tibia	Stromal cell-derived factor-1	Enhanced recruitment of endothelial progenitor cells to distraction gap
Geiger et al.[109]	Rabbit radius	VEGF	Increased vascularity and bone formation in distraction gap
Chan et al.[131,132]	Rabbit tibia	Low-intensity pulsed ultrasound	Dose-dependent callus formation and accelerated bone remodeling
Shimazaki et al.[157]	Rabbit tibia	Low-intensity pulsed ultrasound	Accelerated bone maturation
Sakurakichi et al.[133]	Rabbit tibia	Low-intensity pulsed ultrasound	Increased osteogenic cell differentiation
El-Hakim et al.[137]	Goat mandible	Electrical stimulation	New bone formation and increased mechanical strength of union
Siwach et al.[148], Goel et al.[149]	Human fracture nonunion	Bone marrow mesenchymal stem cells	90% bone union
Peterson et al.[150]	Rat femoral defect	Bone marrow mesenchymal stem cells	Defect healing acceleration
Quarto et al.[151]	Human bone defects	Bone marrow stromal cells	Repair of defect
Lee et al.[158]	Human tibial lengthening	Bone marrow aspirate concentrate and platelet-rich plasma	Small advantage in bone-healing at cortex

The injection of NGF yielded significant recovery of peripheral nerve function ($p < 0.05$) in a study of rabbit mandibular distraction osteogenesis and offers a potential solution to neurologic complications[75].

Locally, a vast range of molecules, including growth factors, and cells have been applied to distraction gaps to

enhance bone-healing. The application of BMP-2 and BMP-7 has been studied in both rat and rabbit distraction osteogenesis models. Both factors have reproducibly promoted bone regeneration at normal and rapid distraction rates[80-85]. BMPs have also been compared with treatment with TGF-β or IGF-1 to explore osteoinduction potential; however, TGF-β and IGF-1 do not contribute significantly to osseous regenerate as lone augmentation factors[86]. TGF-β1 treatment alone has shown no conclusive benefit in animal models[87]. Combining BMP-2 with Nel-like protein 1 (NELL-1), a secretory growth factor, also enhanced the action of BMP-2 as measured by tibial peak loads in a rabbit model[88].

Platelet-rich plasma, rich in growth factors such as TGF-β and platelet-derived growth factor (PDGF), has gained considerable attention in bone-healing literature. Although platelet-rich plasma has been shown to shorten fracture-healing time, results remain controversial[89-91]. In a study of rabbit tibial distraction osteogenesis, the injection of platelet-rich plasma enhanced the consolidation phase of bone regenerate[92]. Similar results have been shown in rat tibiae; however, results may be affected by platelet concentrations or the content of thrombin and thrombin-related peptide[93-95]. PDGF specifically enhances proliferation of mesenchymal cells and angiogenesis, among other functions. Application of PDGF alone to the distraction site of rat femoral osteotomies was sufficient to demonstrate enhanced bone-healing[96]. Local platelet-rich plasma injections in patients undergoing limb lengthening may significantly shorten the necessary treatment time ($p = 0.0412$)[97,98]. Platelet-rich plasma combined with mesenchymal osteoblast-like stem cells expanded in culture have been used clinically in three patients undergoing limb lengthening, achieving a mean healing index time of 23.0 days/cm (range, 18.8 to 26.9 days/cm) bilaterally with minimal complications[97,98].

In cases of bone defects (as in segmental defects due to trauma or bone resection after osteomyelitis or tumor), bone can be regenerated with use of bone transport. This method presents an alternative to traditional grafting techniques and avoids the difficulties associated with allografts. In this technique, bone adjacent to the defect is osteotomized and is subjected to distraction osteogenesis to close the defect[99,100]. Once the bone segment traverses the defect, healing the docking site presents a unique challenge because it infrequently spontaneously heals, more commonly forming a fibrocartilaginous nonunion[101]. A second percutaneous osteotomy to stimulate callus formation has been described, as well as removal of the interposed fibrous tissue to recapitulate a fresh fracture site[101]. In cases of poor contact, bone-grafting may be necessary[102,103]. BMPs have also been applied to stimulate the docking site, with variable success[104-106].

The rate of distraction is an important clinical consideration. Greater distraction rates result in increased mechanical strain within the distraction gap and decreased time for molecular signaling and cell migration. As patient age and comorbidities (for example, diabetes or smoking status) increase, so does the time necessary to heal; pediatric and healthy populations may require faster distraction rates (1 to 1.5 mm/day) to maintain osteogenic potential within the distraction gap and to avoid premature consolidation[6,107]. In high-speed distraction models (distraction rates exceeding 2 mm/day), the failure of callus formation may be due to the unsuccessful recruitment of bone marrow endothelial cells to the osteotomy site. The local application of stromal cell-derived factor-1, a cytokine crucial to angiogenesis, improved the recruitment of bone marrow endothelial cells and callus formation[108]. In a similar attempt to promote angiogenesis, Geiger et al. showed that direct application of VEGF-encoding plasmids coated on a collagen sponge increased vessel formation by twofold to threefold at six weeks, followed by more robust bone formation[109]. By increasing angiogenesis, the oxygen tension within the distraction gap can be restored to favor osteoblast lineage differentiation and thus predispose to faster bone deposition clinically.

Mechanical Enhancement

Mechanical enhancement may also be employed to affect cellular behavior. In cases of poor callus formation, the distraction regimen may be changed to delay distraction or may utilize compression followed by distraction (accordion maneuver) to increase osteogenesis[110,111]. However, slow distraction has shown distinct effects on cell migration, proliferation, and differentiation due to varied cell and extracellular matrix densities as well as cell gradients[112-114].

The classic external fixator has been modified in various ways to influence bone formation. For example, external fixation with greater stability yielded enhanced bone formation[115-117]. The insertion of an intramedullary wire in a dog model of distraction osteogenesis resulted in stimulation of the ossification processes, accelerated bone union, and earlier marrow cavitation[118]. The technique of lengthening over a nail is attractive clinically because, overall, external fixation duration is decreased and the intramedullary nail protects the regenerate from fracture[119-121]. This technique may also decrease rates of axial malalignment and callus subsidence[122]. In a comparison between external fixation alone and external fixation combined with intramedullary nailing in tibial defects, although rates of nonunion, deformity, limb-length discrepancy, and functional results were similar, there was a greater rate of deep intramedullary infection in the combined treatment group if lengthening was >9 cm[123]. In a study of twenty-one patients (twenty-two femoral lengthenings), rates of infection of up to 22% with this technique have been reported[124]. However, if an intramedullary nail is already in place and lengthening is clinically necessary, the time needed for external fixation is substantially reduced because of robust regenerate formation and consolidation[125-127]. Furthermore, lengthening over a nail is

likely associated with fewer complications than an intramedullary skeletal kinetic distractor[128]. Lengthening and then nailing, an integrated fixation technique that avoids concomitant internal and external fixation, results in a shorter time needed in external fixation, lower rates of infection, and enhanced rates of bone-healing, perhaps due to disruption of the regenerate by reaming and subsequent inflammatory and osteoinductive events[129]. Lengthening over a plate has also shown promising results and can be used in skeletally immature patients. In a case series of sixteen patients, Oh et al. showed tibial lengthening with a submuscular plate was reliable and had good to excellent functional results in an adolescent population[130]. Although the mechanisms of mechanical augmentation remain incompletely understood, clinical experience is that intramedullary and plating techniques appear to accelerate healing in distraction osteogenesis and to decrease external fixation duration.

Several commercial mechanical adjuncts have also demonstrated promise in enhancing regenerate formation. Low-intensity pulsed ultrasound has demonstrated a dose-dependent effect on callus formation in distraction osteogenesis, with greater apposition rate, mechanical strength, and bone mineral density following consolidation[131-133]. In fracture repair, ultrasound decreases healing time, which may be due to mimicry of a fluid-induced shear flow milieu, thus increasing cellular bone repair functions[134]. The mechanism of action of low-intensity pulsed ultrasound is complex, implicating Runt-related transcription factor 2 (RUNX2), osteocalcin, alkaline phosphatase, VEGF, and matrix metalloproteinase-13[135,136].

Similarly, the mechanism by which electrical stimulation affects healing in distraction osteogenesis has not yet been elucidated, although it has shown promise in enhancing regenerate bone quality and increased bone surface area[137]. Electrical activity may change oxygen tension or alter cell membrane potentials, thus stimulating osteogenesis[138]. Electrical current may also stimulate undifferentiated mesenchymal cells in the bone marrow to differentiate into osteoblasts[139].

Cellular Therapy

Mesenchymal stem cell transplantation has shown considerable promise in accelerating bone formation in distraction osteogenesis[140,141]. Ilizarov demonstrated bone marrow involvement in bone formation in canine tibiae in 1989[115]. Autologous bone marrow stem cell transplantation into the distraction gap produces bone regeneration promoting the consolidation period and may offer a solution for defect repair or irradiated bone[142]. The success of this approach is likely dependent on the number and concentration of progenitor cells injected into the distraction gap, as evidence suggests nonunions respond in a dose-dependent manner to progenitor cell injection[143,144]. The role of mesenchymal stem cells in bone repair and regeneration is still under robust investigation[145], and genetic modification of mesenchymal stem cells may further enhance bone repair[146]. In many cases, mesenchymal stem cells appear to differentiate toward the local cell populations because of the microenvironment[147]. In two distinct case series of more than sixty patients, almost 90% of cases of nonunion treated with transplanted bone marrow mesenchymal stem cells resulted in bone union[148,149]. Adipose-derived mesenchymal stem cells transplanted into a large rat femoral defect model resulted in defect healing acceleration at eight weeks[150]. Quarto et al. showed repair of large bone defects in patients with use of autologous bone marrow stromal cells[151]. Bone marrow aspirate concentrate has been established as a novel strategy for bone defect treatment after posttraumatic bone loss[151,152]. In a study of twenty-two patients undergoing bilateral tibial lengthening, patients were unilaterally injected with bone marrow aspirate concentrate and platelet-rich plasma and demonstrated a small advantage in bone-healing at the cortex during distraction osteogenesis[153]. Callus shape and type were not different between the groups. These results, taken together, suggest that mesenchymal stem cells, derived from either adipose tissue or bone marrow, are likely to augment the regenerate in the distraction gap and to result in improved clinical outcomes. Importantly, autologous mesenchymal stem cell transplantation is safe, and some methods such as bone marrow aspirate concentrate (BMAC) are extremely efficient and cost-effective.

Conclusions

The complex mechanisms governing distraction osteogenesis healing are still under robust investigation. Three distinct phases of distraction osteogenesis occur: latency, distraction, and consolidation, during which discrete molecular cascades are induced. Healing within the distraction gap is distinct from fracture-healing. Oxygen tension, angiogenesis, cell differentiation, and, ultimately, callus deposition and bone remodeling reflect a delicate physiologic balance in each phase.

The application of both systemic and local factors may improve healing in distraction osteogenesis and may augment the osteogenic potential of pluripotent tissues in the distraction gap. Although BMP-2 and BMP-7 have shown promising effects on bone formation, they may be further augmented with other growth factors, including those found in platelet-rich plasma and proteins found in the extracellular matrix. Mesenchymal stem cell transplantation has shown promising results for regeneration of bone in distraction osteogenesis; however, genetic manipulation and the ideal preparation and timing of injection are still under investigation.

Augmenting osteogenesis to allow for high-speed distraction must be carefully considered clinically. Although prolonged time in an external fixator increases the risk of pin-site infection and other complications, slower distraction times also allow soft-tissue and

nerve accommodation. Faster rates put patients at risk for pain and neuropathy, due to increased tension on nerves, which may result in denervation as well as impaired bone regeneration in distraction osteogenesis[154]. Although osseous union in rapid distraction may be possible with the addition of enhancements, clinical tolerance without concurrent soft-tissue compliance may be limited.

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