New developments and insights learned from distraction osteogenesis

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Purpose of review

Distraction osteogenesis (DO) techniques have been developed and practiced in orthopaedics and craniofacial surgery with outstanding clinical outcomes. This review discusses recent advances in understanding the basic biologic mechanisms of DO, new methods of assessing and promoting bone consolidation in DO, and new clinical applications of DO.

Recent findings

Many genes are upregulated or downregulated in bone cells responding to mechanical stimulation. The changing patterns of BMPs expression and apoptosis may regulate bone regeneration in DO. High magnitude strain promotes bone remodeling, whereas low magnitude stain stimulates osteogenesis. DO not only increases local angiogenesis, but also leads to increased expressions of VEGF and its receptors systemically. Routine radiography remains the most cost-effective imaging technique to follow all aspects of the regenerate, followed by ultrasound, mechanical testing, DEXA and QCT. Minimal noninvasive means of intervention for promoting bone consolidation are preferable, such as weight-bearing exercise, ultrasound, and electromagnetic stimulation; whereas systemic administration of anabolic agents and hormones may also be useful and local application of growth factors such as BMPs remains the last resort. New applications of DO have been extended into treating craniofacial deformities and vascular diseases, but more basic and clinical researches are needed.

Summary

Distraction osteogenesis techniques have a wider implication in understanding the human body's self-repair and self-regeneration potentials and its new clinical applications are to be extended to functional tissue engineering, management of soft-tissue repair, and treatment of vascular diseases and others.

Keywords

distraction osteogenesis, mechanical stimulation, bone consolidation, mandibular distraction osteogenesis, vascular diseases

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Abbreviations

bFGF	basic fibroblast growth factor
BMP	bone morphogenic protein
COX-2	cyclooxygenase-2
DEXA	dual-energy x-ray absorptiometry
DO	distraction osteogenesis
IL-1β	interleukin 1 beta
OP-1	osteogenic protein-1
PGE_2	prostaglandin E2
QCT	quantitative computerized tomography
rhBMP	recombinant human bone morphogenic protein
VEGF	vascular endothelial growth factor

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Introduction

Distraction osteogenesis (DO) techniques have been widely accepted and practiced in orthopaedics, traumatology, and craniofacial surgery over the past two decades; using DO methods, many previously untreatable conditions have been successfully managed with outstanding clinical outcomes [1,2,3•,4,5,6••,7,8]. Although the biologic mechanisms of DO are still not yet fully defined, it is generally accepted that mechanical stimulation is the key in promoting and maintaining tissues' regenerating capacities. This review discusses recent advances in understanding the basic biologic mechanisms of DO, new methods of assessing and promoting bone consolidation during DO treatments, and its potential new clinical applications.

Biologic mechanisms of distraction osteogenesis

Many genes have been found being upregulated or downregulated in the bone cells responding to mechanical stimulation in previous studies [9]. Recently, the nuclear proto-oncogene c-fos and c-jun were found to be upregulated at early stages of DO [10]. As Fos- and Jun-related genes were related to mechanotransduction and embryonic bone development, their strong expressions during DO provide further evidence to support Ilizarov's hypothesis that DO resembles some aspects of embryonic development. A recent study demonstrates that even Schwann cells retain ability to synthesize myelin during gradual nerve elongation [11•]. The expression

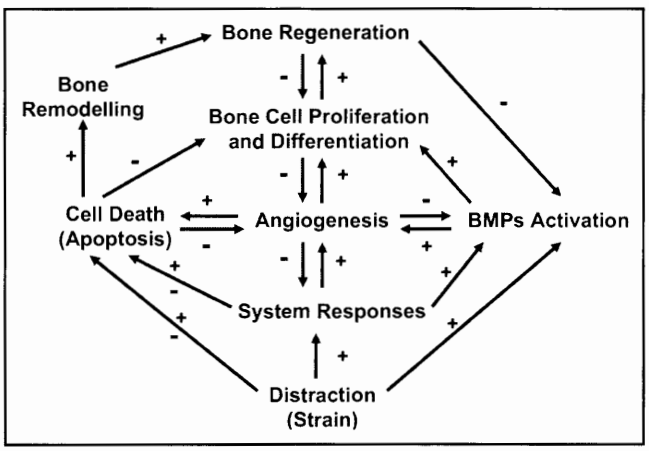
pattern of BMPs during (mandibular) bone distraction is similar to that during long bone distraction [12•]. The BMPs (2, 4, 5, 6, and 7) are expressed continuously from the beginning of distraction until 2 weeks after the completion of DO. BMP-3, which is mainly responsible for halting bone growth at appropriate sites and times, is not detected during DO but is strongly expressed at 1 to 2 weeks during consolidation phase [12•], suggesting BMPs may regulate or control the balance of bone formation and remodeling during DO. During DO, new bone forms and undergoes rapid remodeling and apoptosis may be one of the regulatory mechanisms governing the removal of the redundant callus. The localization of apoptotic cells at the different regions of the regenerate, accompanied by the osteoclast activities, suggest that apoptosis is closely related to bone formation and remodeling during DO [13•].

Mechanical signals play an integral role in bone homeostasis. Low magnitude of tensile stain (2–8% equibiaxial strain) in the tissues have anti-inflammatory effects and inhibit proinflammatory gene expression (such as IL-1β and COX-2), whereas tensile stain of high magnitude (15% equibiaxial strain) induces proinflammatory gene expression, rapidly upregulated COX-2 mRNA expression, and PGE_2 synthesis [14••]. Several studies have suggested that growth factor signaling is also involved in the transduction of mechanical stimuli; for example, epidermal growth factor receptor expression is upregulated in osteoblastic cells under fluid flow [9]. Taken together, these observations reveal an important mechanism that bone resorption may occur in a field experiencing high magnitudes of strain and bone formation results in fields exposed to physiologic or low magnitude of strain. This may also explain the stimulatory effects on bone regeneration/consolidation by weight- bearing exercise, pulsed electromagnetic field stimulation, ultrasound, and short-wave treatment.

It is well documented that DO is a vascular-dependent process. DO stimulate the production of angiogenic factors, such as VEGF and bFGF in the newly formed bones [15]. DO results not only in increased local expression of VEGF and its receptors at the site of distraction gaps, but also leads to increased expressions of VEGF and its receptors levels in distant muscle sides [16•], suggesting that DO induces systemic responses, such as releasing growth factors, cytokines, hormones, and stem cells that promote healing [17•]. The feedback cycles of the biologic cascades induced by DO are summarized in Figure 1.

As to the source of bone-forming progenitors during DO, many believe that the periosteum and bone marrow are the main contributors. In a clinical study on the behavior of periosteum during DO [18•], metal markers were inserted into periosteum of patients who underwent DO;

Figure 1. Biologic cascades of distraction osteogenesis



The diagram summarizes the regulatory and feedback cycles of biologic cascades involved in distraction osteogenesis. All factors in this diagram are interdependent and have positive (+) or negative (–) feedback cycles and effect on each other.

the authors confirmed the importance of periosteum preservation (its presence as a continuous sleeve) for successful DO treatment. In most cases, the periosteum acts as an elastic sleeve surrounding the newly formed bone, and the site of attachment between sleeve and cortex is established during early phases of lengthening, and hardly changes position at later stages of DO [18•]. Poor quality periosteum at operation may indicate slow bone regeneration or poor regenerate quality. With appropriate soft-tissue conditions and mechanical managements, the quality of bone regeneration is not usually a clinical issue. Even chemotherapy prior to DO treatment does not impair bone regeneration [19], which suggests DO treatment is unique in mobilizing and promoting the body's repair and regenerative potentials.

Monitoring bone quality in distraction osteogenesis

Distraction osteogenesis is a lengthy procedure and the healing index, defined as the time needed for each linear centimeter of new bone to form and mature to maintain its structure after fixator removal, ranges from 20 days to 4 to 5 months depending on patient age, bone location, total lengthening, and surgical managements [3•]. Past research has also suggested that long duration of DO treatment can have negative impact upon the physical and psychological well being of patients, particularly the young person. However, a recent study in young people with DO treatment has suggested that with proper support and education, (young) patients can tolerate DO treatment without sustained adverse psychological impact [20].

Noninvasive imaging of DO is crucial to make clinical decisions for optimal outcomes and minimizing risks of fixator removal. Although orthogonal routine radiography

remains the most cost-effective imaging technique to follow all aspect of the regenerate [3•], it is not reliable to predict bony union or the quality or quantity of the regenerating bone, since an estimated 40% increase in radiodensity is needed to visualize a radiologic change, and radiographic changes did not correlate to mechanical stiffness [21•]. Supplemental techniques including mechanical testing for bone strength and stiffness, DEXA for bone mineral density, QCT for density and cortical continuity, ultrasound for cyst detection, and Doppler or angiography for assessing local blood flow and vascularity have all been used clinically. Among them, ultrasound is reported to be a useful and accurate method to evaluate bone fill in DO [22•,23]. The advantages of using ultrasound include: the minimal expense, no metal artefact and radiation exposure, and reduced number of serial radiographs required [22•]. However, the facilities for ultrasound follow-up must be developed with an experienced radiologist and it is only recommended where this possibility exists [23]. Moreover, the mechanical stiffness of distraction regenerate does not always correlate with the plain radiographic and ultrasound data [21•]; even when radiographic consolidation of the distraction regenerate is observed, the literature recommends waiting for 2 extra months for safely removing the external fixation [24•]. Therefore, the clinical decision of fixator removal must be made on a case-by-case basis by experienced clinicians.

Promoting bone consolidation in distraction osteogenesis

Although DO has revolutionized the treatment of many orthopaedic disorders, one of the problems of this technique is the long waiting period for newly formed bone to consolidate, which can cause considerable morbidity to the patients, such as pin-track infection, delayed consolidation, and discomfort caused by the bulky frame [24•]. Various approaches have been tested to enhance bone formation during DO, as summarized in Table 1.

Table 1. Approaches and factors promote bone regeneration during distraction osteogenesis

Mechanical
• Weight-bearing (mechanical compression)
• Ultrasound (low velocity)
• Electromagnetic field stimulation
• Electrical currents stimulation
• Short-waves treatment
Biomaterials/cells
• Calcium sulfate
• Tri-calcium phosphates
• Autologous bone grafts and allografts
• Chitosan and other biopolymers
• Osteoblastic cells
• Bone marrow extracts
• Platelets
Hormones/anabolic and antiresportive agents
• Growth hormone
• PTH
• Estrogen
• PGE_2
• Bisphosphonates
• Zoledronic acid
Biomolecules/growth factors
• BMP-2/BMP-4
• BMP-7/OP-1
• VEGF
• FGF-2
• TGF-β
• Others

Mechanical stimulation by controlled weight-bearing exercises promotes bone consolidation by stimulating angiogenesis, and the newly formed vessels in the periosteal region are more sensitive to mechanical stimulation than the endosteal vessels [25]. This again suggests the importance of periosteum preservation and postoperative physiotherapy managements. Pulsed electromagnetic field stimulation may be a safe and cost-effective way of promoting bone consolidation in DO, since electromagnetic field stimulation increases callus formation but does not affect the callus remodeling phase [26], and electromagnetic stimulation can reduce the latency period, from 7 to 10 days to 1 day, following osteotomy without compromising overall bone regeneration of DO [27].

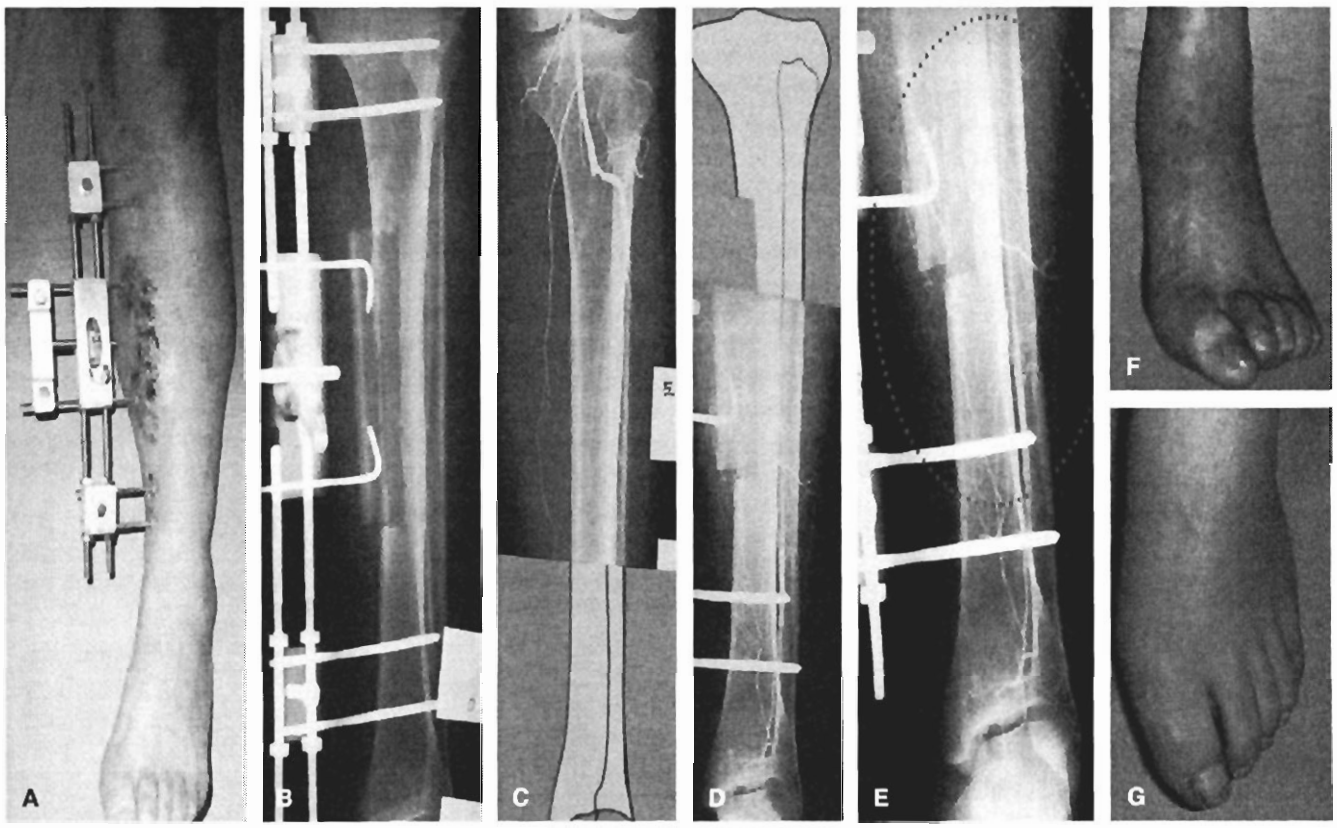
Some studies suggest that early conversion from external to internal fixation may be an alternative for reducing complications of DO [24•]. After speedy lengthening by external fixator, secondary external fixation accompanied with bone marrow cells with biomaterials, autologous bone grafts or allografts has achieved good clinical outcomes [24•]. However, this approach contradicts the basic principles of DO techniques defined by Ilizarov [1], in that a rigid fixation coupled with careful corticotomies (preservation of intramedullary blood supply) and distraction at a rate of 1 mm/d in four or more steps daily will lead to a guaranteed successful DO treatment. But in clinical practice, these basic principles may not be easy or possible to follow and the surgeons must be open-minded and prepared to test out new methods of improving DO treatment.

Systemic administration of anabolic agents and hormones to promote bone regeneration is an appealing strategy. Growth hormone has shown to promote early bone consolidation when given a daily subcutaneous injection of 1 IU/kg in a dog DO model [28•], and bone mechanical strength increased three times in the growth hormone group than in the control group. Prostaglandins are anabolic agents *in vitro*, but they can not be used *in vivo* due to its gut-intestinal side effects [29•]. Recent study into the PGE_2 receptor, such as EP2 receptor-selective prostaglandin E2 agonist, may lead to a new class of anabolic agents that can be administrated locally and systemically to stimulate osteogenesis and fracture healing, but their clinical usefulness is yet to be tested [29•]. Antiresorptive agents such as bisphosphonates have been reported to have positive effect on fracture

healing. A recent study showed that in a rabbit model of leg lengthening, systemic administration of zoledronic acid (0.1 mg/kg) once or twice increased distraction to regenerate volume, mineralization, and strength [30•], suggesting bisphosphonates may have an anabolic effect in addition to its antiresorptive effects. However, a dose-related negative effect of zoledronic acid on the longitudinal growth of young rabbits has been noted [30•]; therefore, it may not be safe to give bisphosphonates such as zoledronic acid to children undergoing DO treatment. In a similar rabbit model of DO, systemic administration of 10 IU salmon calcitonin for the entire duration of distraction did not enhance the rate of bone consolidation [31], suggesting administrating antiresorptive agent alone may not benefit bone consolidation in DO.

Local application of BMPs to promote fracture healing and spine fusion has become an accepted clinical alternative. In a rabbit DO model with a rapid rate of lengthening (2 mm/d), single application of rhBMP-2 (75 μg) by injection or implantation at the end of the distraction period has significantly enhanced bone maturation and bone consolidation [32]. In contrast, when rabbits were lengthening at a slow rate (1 mm/d), injection of OP-1 (BMP-7) from 80 to 2000 μg did not show significant enhancing effects on bone maturation and consolidation [33•], suggesting additional growth factors are not normally needed during DO under normal circumstances. Since DO is a vascular-dependent process, the angiogenic factors may also have positive effects on bone regeneration during DO. However, a recent study in a rabbit DO model has revealed that locally applied vascular endothelial growth factor (VEGF) and VEGF inhibitor had no effect on blood flow, blood vessel formation, and the quality of bone regeneration in the distraction gap [34•], suggesting there may be different biologic properties and regulatory pathways of the vasculature in the native and regenerating bone during DO. In summary, additional growth factors such as BMPs and VEGFs may not promote bone regeneration any further during DO under normal circumstances, where bone regeneration is already at its optimal speeds. But additional growth factors may enhance bone regeneration and consolidation in conditions where bone argumentation is clearly needed.

Figure 2. Treatment of vascular disorder by distraction osteogenesis



(**A**) A patient with thromboangiitis obliterans had an external fixator on the tibia. (**B**) Radiograph shows that a piece of cortex resulted from a longitudinal cortical osteotomy, was attached the external fixator. (**C**) Angiography before operation shows that small blood vessels beneath the arteriae poplitea were not detectable in the diseased leg. (**D** and **E**) At 25 days after transverse bone transport, angiography shows that new vascular network has developed in the treated leg, with many new small vessels. (**F**) Before treatment, the foot of the diseased leg had appearances of chronic ischemia, with dry skins, nails, and focal ulcers. (**G**) 25 days after DO treatment completed, the foot regained its circulation with improved appearances of skins and nails, and the chronic ulcers started healing up. [All photos are courtesy of Dr. Long Qu, Beijing Bone Lengthening and Bone Transport Center, Beijing Aerospace Hai-Ying Medical Center, Beijing, PR China.]

New clinical applications of distraction osteogenesis

In addition to its well-known applications in orthopaedics and traumatology, DO techniques have also been widely accepted and performed in craniofacial surgery [2,4,5,6••,7,8]. Mandibular DO is becoming the first choice of treatment for patients with hypoplastic mandibles [5,8]. Micrognathia accompanying obstructive sleep apnea syndrome, which is a difficult clinical problem with mandible deformities (shortening) and temporomandibular joint ankylosis, usually results in airway space narrowing [6••]. Mandibular DO has achieved excellent clinical outcome for treating micrognathia accompanying obstructive sleep apnea syndrome with no age limit of the patients [6••]. DO in craniofacial surgery has been developed rapidly and will advance the management of complex craniofacial anomalies, but at the present, its procedures have not been fully established in comparison to its applications in orthopaedics, and more basic and clinical studies are needed to perfect DO techniques in craniofacial surgery [4].

Another exciting new development in DO application is to treat vascular conditions such as peripheral chronic artery obliterations [35]. Qu *et al.* [36] have reported treating patients with thromboarteritis thromboangiitis obliterans successfully by transverse tibial cortex transport (Fig. 2). In that process, a longitudinal cortical osteotomy was performed splitting a piece of cortex (120 mm × 20 mm), which was fixed to an external fixator. After a 5-day latency period, the split cortex was lengthened at 1 mm/d for 20 days transversely (Fig. 2A, B). From the beginning of the bone transport, the symptoms of cool sensation of leg, the tingling of feet, and the intermittent claudication disappeared gradually in all the patients. On angiography, 25 days after the bone transport completed, a richer vascular network developed in the treated leg (Fig. 2 D, E) compared with before the treatment (Fig. 2C). The increased blood flow and vasculature after transverse tibial lengthening (thickening) by the Ilizarov method has also been reported in a dog DO model [36], where changes in blood flow and vasculature were most pronounced in the distraction leg and sustained a long-lasting stimulatory effect of promoting circulation in the treated extremity [36]. The positive effects of DO on angiogenesis, vasculature formation and blood flow of the treated extremity suggest that DO may be an invaluable tool to treat vascular or circulation related disorders, such as diabetic ulcers, chronic artery obliterations, avascular necrosis of femoral heads, and chronic soft-tissue infections.

Conclusion

Many genes are upregulated or downregulated in bone cells responding to mechanical stimulation. The changing patterns of BMPs expression and apoptosis may regulate bone regeneration in DO. High magnitude strain promotes bone remodeling, whereas low magnitude stain stimulates osteogenesis. DO not only increases local angiogenesis, but also leads to increased expressions of VEGF and its receptors systemically. Routine radiography remains the most cost-effective imaging technique to follow all aspect of the regenerate, followed by ultrasound, mechanical testing, DEXA, and QCT.

Under normal circumstances with adequate support of postoperative physiotherapy, DO treatment has good clinical outcome and needs no additional intervention(s). However, if bone consolidation becomes a clinical concern, minimal noninvasive means of intervention for promoting bone consolidation are preferable, such as weight-bearing exercise, ultrasound, and electromagnetic stimulation. Systemic administration of anabolic agents and hormones may also be useful and local application of growth factors such as BMPs remains the last resort, as their working mechanisms in DO are still not clearly known and are expensive.

Being a great surgical technique for tissue repair and regeneration, DO also has a wider implication in understanding the body's self-repair and self-regeneration potentials, and its new clinical applications are to be extended to functional tissue engineering, management of soft-tissue repair, and treatment of vascular diseases and others.

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