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The Molecular and Cellular Events That Take Place during Craniofacial Distraction Osteogenesis

Adi Rachmiel, DMD, PhD
Yoav Leiser, DMD, PhD

Summary: Gradual bone lengthening using distraction osteogenesis principles is the gold standard for the treatment of hypoplastic facial bones. However, the long treatment time is a major disadvantage of the lengthening procedures. The aim of this study is to review the current literature and summarize the cellular and molecular events occurring during membranous craniofacial distraction osteogenesis. Mechanical stimulation by distraction induces biological responses of skeletal regeneration that is accomplished by a cascade of biological processes that may include differentiation of pluripotential tissue, angiogenesis, osteogenesis, mineralization, and remodeling. There are complex interactions between bone-forming osteoblasts and other cells present within the bone microenvironment, particularly vascular endothelial cells that may be pivotal members of a complex interactive communication network in bone. Studies have implicated number of cytokines that are intimately involved in the regulation of bone synthesis and turnover. The gene regulation of numerous cytokines (transforming growth factor-β, bone morphogenetic proteins, insulin-like growth factor-1, and fibroblast growth factor-2) and extracellular matrix proteins (osteonectin, osteopontin) during distraction osteogenesis has been best characterized and discussed. Understanding the biomolecular mechanisms that mediate membranous distraction osteogenesis may guide the development of targeted strategies designed to improve distraction osteogenesis and accelerate bone regeneration that may lead to shorten the treatment duration. *(Plast Reconstr Surg Glob Open 2014;2:e98; doi: 10.1097/GOX.0000000000000043; Published online 23 January 2014.)*

The indications of distraction osteogenesis in craniomaxillofacial field are increasing in the last 2 decades mainly in severe cases of hypoplastic bones and in the treatment of maxillofacial asymmetry as seen in hemifacial microsomia[1–3] or lengthening of severely hypoplastic mandible as seen in Pierre Robin or Treacher Collins syndromes, resulting in obstructive sleep apnea.[4,5] Other indications of distraction are the treatment of hypoplastic maxilla in cleft palate patients.[4,6,7] Distraction osteogenesis of facial membranous bones provides an excellent in vivo system of membranous bone formation. In this system, bone is generated by stretching a callus that develops following osteotomy of midfacial bone (Fig. 1). Distraction osteogenesis is based on the "tension-stress principle" proposed by Ilizarov.[8,9] The essence of this technique is the gradual distraction of

From the Department of Oral and Maxillofacial Surgery, Rambam Health Care Campus, Haifa, Israel; and The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.
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a fracture callus after osteotomy or corticotomy of the facial skeleton bones with careful preservation of the soft-tissue envelope overlying the bone (Fig. 1). However, the method requires several days of latency period, several weeks for active lengthening, and several months for consolidation until mature lamellar bone is formed for stable results. The extended wearing of the distraction devices several months especially those with external devices is uncomfortable to the patients and may create compliance complications.[10–12]

Mechanical stimulation by distraction induces biological responses of skeletal regeneration that is accomplished by a cascade of biological processes that may include differentiation of pluripotential tissue, angiogenesis, osteogenesis, mineralization, and remodeling.[13,14] Our group,[14] using immunohistochemical analysis and electron microscopy, characterized the bone formed and angiogenesis processes during the membranous midfacial distraction and also following the consolidation period and defines the characterization of the new bone in the distracted area (Fig. 2). It was found that as a result of the distraction force, a pool of undifferentiated mesenchyme-like cells is created with osteogenic potential which in turn triggers capillary formation. The new bone trabeculae begin to form between 5 and 10 days following the beginning of the distraction process (Fig. 3). These trabeculae soon become aligned with the osteoblasts and continue to grow as long as distraction forces are applied (Fig. 4). Vascular formation is intimately associated with bone formation during distraction osteogenesis.[14] There are complex interactions between the osteoblasts, the

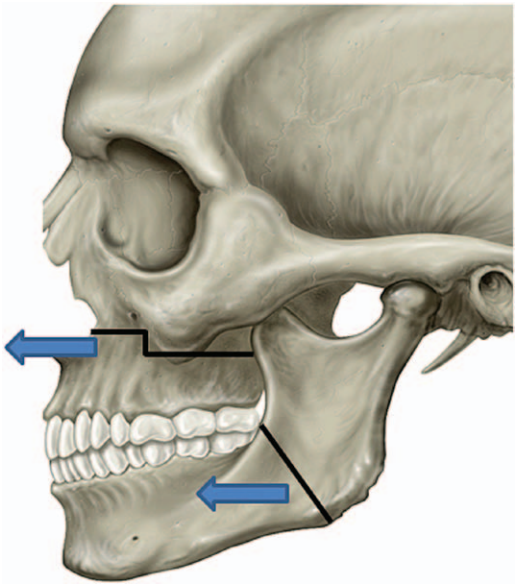

Fig .1. Illustration demonstrates the maxillary and mandibular osteotomy before the distraction. The arrows demonstrate the direction of the lengthening.

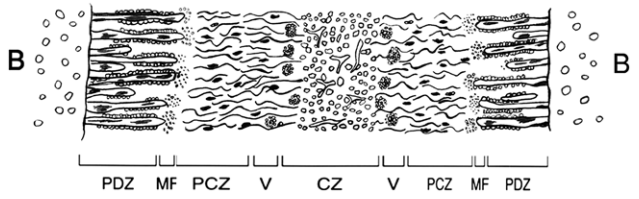

Fig. 2. Schematic drawing of bone formation and vasculogenesis during midface maxillary distraction. During the distraction period, the regenerated tissue can be divided into 3 zones and 2 transitional areas: CZ, central zone (mesenchymal area or proliferative area); PCZ, 2 paracentral zones (fibroblastic area or collagenous area, on both sides of the central zone); PDZ, distal and proximal zones (trabecular area or mineralization area); MF, mineralization front area (transitional area); V, vasculogenesis area (transitional area). B represents the 2 parts of the old bone that became apart after the osteotomy and distraction.

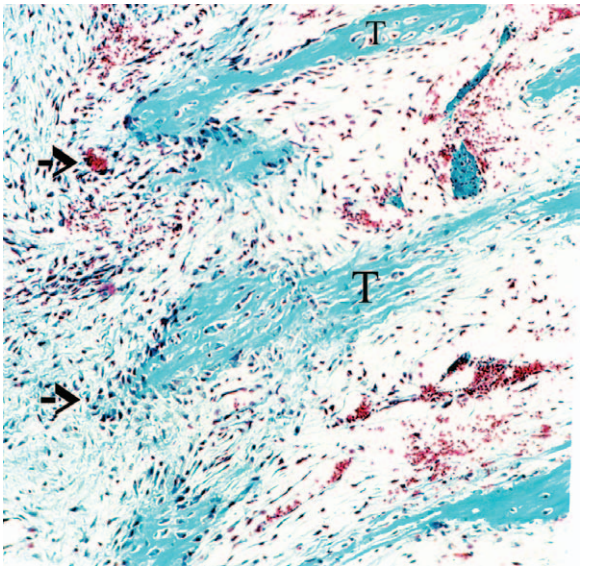

Fig. 3. Recruitment of preosteoblasts (arrows) to the newly formed delicate trabeculae (T) (Masson trichrome; original magnification, 100×).

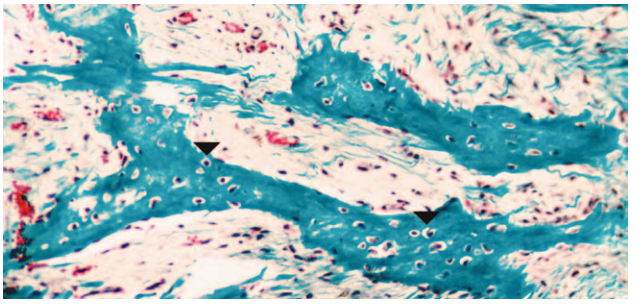

Fig. 4. The bony trabeculae (arrows) during the consolidation period become thicker toward lamellar bone (Masson trichrome; original magnification, 100×).

bone-forming cells, and other cells present within the bone microenvironment, particularly vascular endothelial cells that may be pivotal members of a complex interactive communication network in bone.[14–17] Past studies have implicated a number of cytokines that are involved in the regulation of bone synthesis and turnover.[15,16,18,19] The gene regulation of numerous cytokines [including transforming growth factor (TGF)-β, bone morphogenetic protein (BMP), insulin-like growth factor (IGF)-1, and fibroblast growth factor (FGF)-2] and extracellular matrix proteins (osteonectin and osteopontin) during distraction osteogenesis have been best characterized and are discussed later in this article. Understanding the biomolecular mechanisms that mediate membranous distraction osteogenesis may guide the development of targeted strategies designed to improve distraction osteogenesis and accelerate bone regeneration that may improve the clinical results with better bone quality and quantity and shorten the consolidation time with less relapse following larger bone lengthening as are in severe cases.

EFFECT OF DISTRACTION OSTEOGENESIS ON BONE CELLS

Bone cells respond to mechanical stimulation by gene expression. Lewinson et al[20] have demonstrated that mechanical stimulation of regenerating bone by daily distraction stimulates the expression of early-response genes of the activator protein 1 family of transcription factors. After 15 days of distraction, when bone trabeculae start to form, mostly preosteoblasts and osteoblasts retained c-Fos and c-Jun immunoreactivity. The elevated expression of c-Jun and c-Fos is related to mechanical stimulation due to the distraction forces.[20]

Bone formation by osteoblasts is essential not only for skeletal growth and bone remodeling but also for bone healing and repair. Several hormones and growth factors that are implicated in the regulation of bone physiology are now known to up-regulate the expression of proteins of the AP-1 complex.[21–25] Several genes coding for bone-associated proteins contain an AP-1 response element in their promoter, including collagen type I, alkaline phosphatase, osteocalcin, collagenase-3, and parathyroid hormone/parathyroid hormone–related peptide receptor.[22,25,26] Moreover, one of the AP-1 proteins, c-Fos, has been implicated in transduction of mechanical stimulation to bone cells.[22,27]

THE ROLE OF PROINFLAMMATORY CYTOKINES IN DISTRACTION OSTEOGENESIS

The expression of proinflammatory cytokines interleukin (IL)-1 and IL-6 is elevated once distraction has started and mechanical strain is applied to the callus (Table 1). During the distraction phase, IL-6 is expressed by the oval cells. The IL-6 released in response to stress contributes to intramembranous ossification by enhancing the differentiation of cells committed to the osteoblastic lineage.[28] During distraction osteogenesis in mouse tibiae, tumor necrosis factor-α messenger RNA levels markedly increased toward the end of consolidation.[21] In addition, the receptor activator of nuclear factor kappa-B ligand/osteoprotegerin expression ratio increased at the beginning of the distraction phase and decreased by the end of consolidation (Table 1). These results are similar to those from another study conducted on mandibular distraction osteogenesis.[29] A comparison of the results sug-

Table 1. The Cellular and Molecular Events Taking Place during Craniomaxillofacial Distraction Osteogenesis

Phase	Cellular Events	Molecular Events
Latency	During the osteotomy, a hematoma is formed, inflammation is initiated by the up-regulation of IL-1 and IL-6, mesenchymal stem cells are recruited by a rise in BMP-2 and BMP-4, and ossification is propagated by a molecular increase of BMP-6 and TGF	IL-1↑, IL-6↑, BMP-4↑, BMP-6↑, TGF-β↑, RANK/OPG↑
Distraction osteogenesis	During the stretching of the callus, a central fibrous interzone is formed; the fibroblasts secrete collagen fibers which align with the vector of elongation; IL-6 is up-regulated, which in turn promotes osteoblastic differentiation during the intramembranous ossification stage; and RANK ligand/OPG ratio is high during this phase, which in turn promotes resorption of the remaining mineralized cartilage formed during the latency phase. BMP-2, BMP-4, BMP-6, TGF-β, IGF-1, and bFGF increase and peak during this phase, which promote bone formation in response to the distraction forces. Neovascularization is initiated by the induction of VEGF and angiopoietin-1 and angiopoietin-2	IL-6↑↑, RANK/OPG↑↑, BMP-2, BMP-4↑↑, BMP-6↑, TGF-β↑↑, bFGF↑↑, VEGF↑↑, angiopoietin-1 and angiopoietin-2↑↑
Consolidation	During this phase, maturation and mineralization of the bone trabeculae are continued, yet BMP-2, BMP-4, and bFGF are gradually down-regulated and remodeling of the newly bone is initiated by the up-regulation of TNF-α	BMP-2↓, BMP-4↓, bFGF↓, TNF-α↑↑

RANK/OPG, receptor activator of nuclear factor kappa-B/osteoprotegerin; TNF, tumor necrosis factor.

gests that the resorption of mineralized cartilage in the external callus areas that form adjacent to the ends of the bone tissues and in the gap during the latency phase of distraction osteogenesis is more dependent on the levels of receptor activator of nuclear factor kappa-B ligand and osteoprotegerin and less affected by other cytokines.[30]

EFFECTS OF DISTRACTION OSTEOGENESIS ON THE PERIOSTEUM AND THE ROLE OF HYPOXIC CHANGES FOLLOWING THE SURGICAL CUT

Tissue hypoxia is caused following soft-tissue injury, post osteotomy, and during distraction forces.[31,32] The hypoxic environment affects cell survival and initiates angiogenesis by a complex and multistep mechanism.[33] This leads to a hypoxic microenvironment of the cells and enhances the expression of various cytokines and growth factors that may regulate angiogenesis and bone remodeling. However, hypoxia has a roll in communication between endothelial cells and osteoblast progenitors during the osteosynthesis and bone remodeling. Following the osteotomy, the formation of trabecular bone occurs under hypoxic conditions.[34] Cell culture models recapitulate events that occur in woven bone synthesis and are carried out using primary osteoblasts, osteoblast precursors such as bone marrow-derived mesenchymal stromal cells, or various osteoblast cell lines. Blengio et al[35] suggest that conditions of hypoxia cause inflammation by tuning the cytokine/chemokine repertoire. During these phases, there is an up-regulation of hypoxia-inducible genes coded for cytokines with a primary role in inflammation and angiogenesis, and they include osteopontin, vascular endothelial growth factor (VEGF), and IL-1. This condition of cell proliferation, angiogenesis, and osteogenesis promotes the formation of fully mature bone in distraction osteogenesis.

Several studies demonstrate the potential of bone formation by the periosteum during distraction.[36,37] One of the mechanisms in distraction osteogenesis is exposure of cells that are provided by the periosteum and that have the ability to transform into osteoblasts.[38] Mesenchymal cells transform into osteoblasts through an appropriate periosteal stimulation, and subperiosteal callus makes the peripheral part of the new forming bone. Although it is accepted that the force applied during distraction osteogenesis has an effect on subperiosteal bone formation, the formation of subperiosteal bone can be obtained also by a distraction on the periosteum.[39–41]

THE ROLE OF BMP IN DISTRACTION OSTEOGENESIS

Bone induction during regenerate ossification is a sequential cascade that includes chemotaxis, mitosis, and differentiation of both bone and cartilage.[42] BMPs purified from demineralized bone matrix of variety of mammalian species[42–45] govern these 3 key steps in new bone formation. BMPs act at an early stage of bone induction (Table 1), and they promote and maintain bone formation. BMPs have a role in enhanced recruitment, proliferation, and differentiation of pluripotent mesenchymal cells at the osteotomy site and become progenitor cells with the potential to form new bone. Differentiated mesenchymal cells may support the differentiation of other precursor cells and may stimulate the production of other growth factors such as TGF-β, FGFs, and IGFs.[46]

The expression of BMP-2 and BMP-4 is strongly enhanced by the application of mechanical strain during the distraction phase. They are produced by osteogenic cells at the primary mineralizing front. Once distraction has stopped, the expression of BMP-2 and BMP-4 gradually disappears.[19,47,48] These BMPs play a role in the proliferation of cells required for the completion of bone healing.[18,47,49] As BMP-2 has osteoinductive properties, the administration of exogenous BMP-2 has been used successfully to shorten the treatment time during distraction osteogenesis by accelerating bone formation during the consolidation stage.[18,50] It has been reported that BMP-7 plays a role similar to that of BMP-2 and BMP-4 in distraction osteogenesis[48]; however, most experiments have detected only weak levels or no expression of BMP-7 during distraction osteogenesis.[19,49,51]

TGF-β

TGF-β follows an increased level of expression that lasts into the distraction phase. It displays diffuse expression throughout the distraction gap.[52] An inverse relationship between TGF-β and osteocalcin has been observed in a canine distraction model, where elevated TGF-β levels were accompanied by lower levels of osteocalcin after the initiation of distraction osteogenesis.[53,54] These observations suggest that TGF-β acts as a suppressor for osteoblast maturation by delaying cellular differentiation during the mineralization stage of distraction.

OTHER MORPHOGENS AND GROWTH FACTORS

IGF-1 and basic FGF (bFGF) are also up-regulated during distraction.[55] bFGF is mainly expressed by cells of osteoblastic lineage and mesenchymal cells on the newly formed trabecular bone.[56] Unlike

bFGF, IGF-1 is diffusely expressed throughout the distraction gap[52] (Table 1).

PLATELET-RICH PLASMA

The term platelet-rich plasma (PRP) refers to different types of platelet concentrates obtained using different techniques.[57] It is believed that PRP contains growth factors and might therefore have biological properties that could enhance the regeneration of certain tissues.[58–61] It was also recently implied to have antimicrobial properties which also contribute to tissue repair and regeneration.[62] It has been demonstrated that the administration of PRP in combination with bone marrow cells during the consolidation phase of distraction osteogenesis enhances the bone healing process.[63–67] PRP can also be an effective scaffold to induce osteogenesis. It was shown experimentally that the combination of mesenchymal stem cells with PRP increases new bone formation, mineralization, and mechanical property compared with the PRP-only group and is more effective for reducing the consolidation period in mandibular distraction osteogenesis.[68,69] Latalski et al[70] demonstrated in humans that injection of PRP can enhance bone healing during limb lengthening by distraction osteogenesis. The main advantage of the use of PRP was seen as a significantly shorter treatment time. The injection of PRP into regenerate bone might be an effective method to shorten treatment time during craniofacial distraction and may lead to better functional outcomes and improved patient satisfaction and compliance.

ROLE OF ANGIOGENIC FACTORS IN DISTRACTION OSTEOGENESIS

During distraction forces, there is an inevitable increase in blood flow, to facilitate a successful induction of new bone regeneration.[71,72] Neovascularization during distraction osteogenesis may be induced by VEGF-A and neuropilin (especially neuropilin 1), an alternative receptor for VEGF. VEGF-A expression was localized mainly to the maturing osteoblasts at the primary mineralizing front and to the osteoclasts.[15] The localization finding of VEGF-A suggests that there is coordination between areas of neovascularization and newly formed bone.[73] Another family of angiogenic factors, the angiopoietins, is also expressed during distraction[74] (Table 1). The temporal appearance of angiopoietin-1 is followed by angiopoietin-2, which in turn is followed by a maximal expression of VEGF-A in the distraction model. Angiopoietin-2 by itself is antagonistic to angiopoietin-1. However, it has been proposed that the combination of angiopoietin-2 and VEGF-A stimulates new vessel formation, enhances the plasticity of existent larger vessels, and contributes to new vessel formation.[73] It has also been reported that the increase in VEGF-A and angiopoietin-1 expression is associated with an up-regulation in the expression of hypoxia-induced factor-1α, which is one of the key transcription factors regulating genes associated with an angiogenic response, such as VEGF-A and angiopoietin-1.[72,73] An optimal angiogenic response has been shown to be directly related to the rate of distraction. Numerous investigators have speculated that it is this characteristic that drives bone formation, through an intramembranous pathway.[17,75] Studies have shown that the regulation of angiogenesis in distraction tissues is associated with much higher levels of hypoxia-inducible factor 1α.[21] The transient up-regulation of hypoxia-inducible factor 1α in response to each round of distraction would suggest that many of the downstream genes that are targets of transcriptional activation of hypoxia-inducible factor 1α, such as VEGF-A, may play a major role in promoting new bone formation during distraction osteogenesis. Both angiogenesis and osteogenesis in distraction osteogenesis were dependent on the activity of both VEGF receptors 1 and 2.[76]

EFFECT OF DISTRACTION OSTEOGENESIS ON TOOTH DEVELOPMENT

Although dental injuries during the distraction phase are a minor disadvantage compared with the vast benefits offered by DO, there are injuries that need to be addressed and correcting these drawbacks might lead to reconsideration of the type of the device and the timing of DO. The majority of injuries that can be seen during the distraction phase include root malformations, hindered tooth development, and the destruction of tooth follicles. Positional changes such as shifts or tilted teeth were also found.[77]

CONCLUSIONS

The distraction force applied to the craniofacial skeleton creates a pool of undifferentiated mesenchyme-like cells with osteogenic potential which in turn trigger the formation of new capillary to the area. New bone trabeculae begin to form between 5 and 10 days following the initiation of the distraction forces, and these trabeculae soon become aligned with osteoblasts and continue to grow as long as the distraction force is applied. The bone formation is intimately dependent on formation of vascular tissue. Inadequate blood supply leads to many of the complications in various postsurgical

bone treatments. The distraction osteogenesis process is driven by the activities of molecular mediators of inflammation, the TGF-β super family of morphogens (BMPs), and mediators of angiogenesis. Understanding the biomolecular mechanisms that mediate membranous distraction osteogenesis may guide the development of targeted strategies that may improve distraction osteogenesis and accelerate the bone regeneration.

Yoav Leiser, DMD, PhD
Department of Oral and Maxillofacial Surgery
Rambam Medical Center
Haifa, Israel
E-mail: y_leiser@rambam.health.gov.il

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