Distraction Osteogenesis: Biologic and Biomechanical Principles

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2.1 A Brief History

Distraction osteogenesis (DO) is a bone-regenerative process in which an osteotomy is followed by gradual distraction of the surrounding vascularized bone segments, with formation of new bone within the distraction gap. This process was first described by Alessandro Codivilla at the turn of the twentieth century [1, 2]. Codivilla demonstrated the ability to lengthen the chronically deformed femur or tibia 3–8 cm following an oblique osteotomy. He did this by applying a 25–30 kg distractive force across a full extremity plaster cast, which was serially and circumferentially cut near the level of deformity. Application of traction occurred only at the time of cast adjustment, causing a gap to form, which was then filled with additional plaster. This frequently resulted in pressure necrosis due to rubbing of the cast against the soft tissues of the leg.

Early limb lengthening attempts were met with complication and poor predictability and thus were not generally accepted before the work of Gavriil Ilizarov in the 1950s and 1960s. An orthopedist in Russia, Ilizarov conducted a series of experiments using the canine tibia to optimize distraction osteogenesis using a multi-pin circumferential external fixator. His advances included determination of optimal pin stability within the fixator, minimization of soft tissue disruption including periosteum preservation to maintain blood supply, demonstration of feasibility of corticotomies rather than osteotomies, determination of ideal latency and activation periods, and a rigorous histologic assessment of the distraction site, including description of the *neo-physis* [3, 4]. Ilizarov applied his findings to limb lengthening operations in over 15,000 patients, but his clinical work was unknown to the Western world until 1980. That year he operated on the Italian explorer, Carlo Mauri, who had a 10-year-old tibial deformity from a skiing accident deemed uncorrectable.

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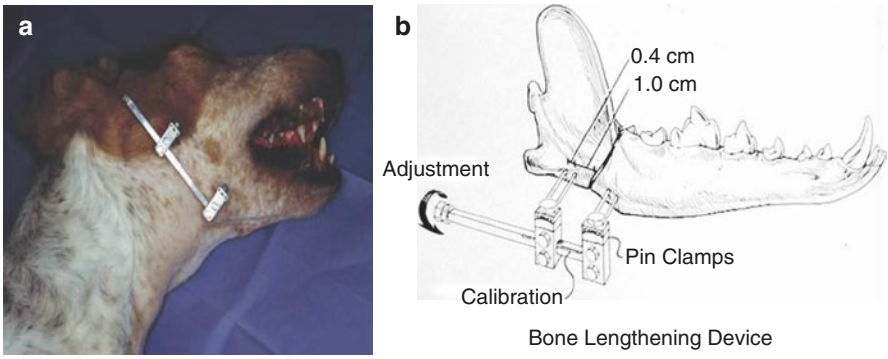

Fig. 2.1 (**a**) Canine with NYU extraoral distraction device. (**b**) Drawing of NYU canine model with osteotomy site and extraoral distraction device

Ilizarov corrected this deformity and pseudoarthrosis using his technique, and upon Mauri's return, Ilizarov was promptly invited to present his work at a conference in Bellagio in 1981. This was the first time Ilizarov spoke outside the Iron Curtain. His technique then spread rapidly throughout Europe and to the US by the late 1980s.

Within the craniofacial skeleton, distraction osteogenesis was first attempted experimentally in 1972 by Snyder [5]. His group surgically shortened one side of a canine mandible and then corrected the resultant crossbite using an external screw-driven distractor device. It was, however, the work of McCarthy and his colleagues at New York University who conducted a series of canine experiments [6, 7] (Fig. 2.1) that resulted in the first human application in 1989, when the mandibular body and ramus were lengthened in four young patients with congenital micrognathia [8]. This report ushered in the era of craniofacial distraction osteogenesis. Subsequent experiments in animal models further demonstrated the utility of distraction osteogenesis for lengthening or expanding the midface [9, 10], zygoma/glat [11], and cranial vault [12, 13]. Based on these studies and others, the use of distraction has since expanded to treat a wide range of congenital anomalies and acquired deformities throughout the craniofacial skeleton.

2.2 Classification of Distraction Osteogenesis

Distraction osteogenesis may be classified based on treatment goal, type of distraction device, anatomic location, or operative approach. When considering the goals of treatment, there are three modalities: (1) pure lengthening procedures, (2) bone segment transportation for correction of defects without lengthening, (3) and corrective distraction osteotomies. Bone lengthening is the most common application and requires a single osteotomy or corticotomy followed by application of distractive forces (Fig. 2.2, *top panel*). Bone transport osteogenesis is used to fill bony defects by advancing adjacent bone or "transport segment" into the gap. One or two transportation segments may be used, depending upon defect size and

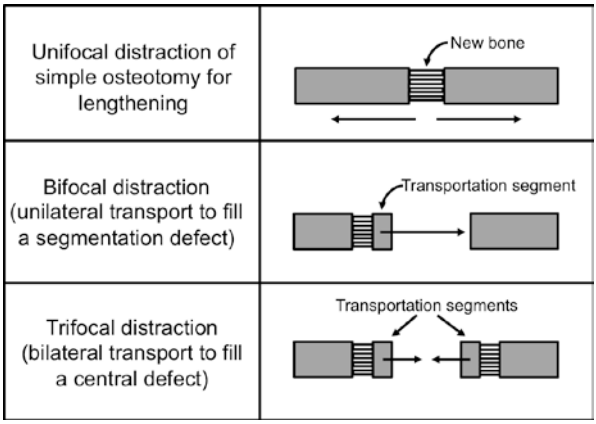

Fig. 2.2 Modalities of distraction osteogenesis

surgeon preference (Fig. 2.2, *middle and bottom panels*). Corrective distraction osteotomies combine bone lengthening with additional movements to correct shortened limbs with varus/valgus or rotational deformities. Both lengthening procedures and bone segment transport modalities are used within the craniofacial skeleton.

Distraction osteogenesis devices may also be classified as either external or internal. Both classic limb lengthening and early mandible distraction utilized external distraction devices. These devices generally have threaded drive shafts, which interface with the osteotomized bone by pin fixation. More recently internal devices have gained in popularity for use in craniofacial applications, particularly for the mandible and cranial vault. These are typically directly applied to the bone via screw fixation in a subperiosteal plane. Internal devices have the advantage of increased rigidity and absence of cumbersome external hardware, but may decrease bone blood supply, as they require greater periosteal dissection and require a separate procedure for device removal. Internal distraction for limb lengthening is now possible using periosteum-sparing telescoping intramedullary nails [14].

Anatomic location is another means of classifying distraction osteogenesis. Within the appendicular skeleton, the procedure is most commonly described within the long bones of the lower extremity (Table 2.1). Within the craniofacial skeleton, distraction osteogenesis was initially applied to the mandible; however, its use has increasing popularity within the maxilla (particularly the alveolus), the midface, and the cranial vault.

A final classification consideration is whether a *trans-sutural* (closed) or *trans-osteotomy* (open) operative approach is used. It is arguable whether closed approaches actually constitute distraction osteogenesis, as no osteotomies are performed; however, they do utilize an activation and consolidation period. A closed approach within the appendicular skeleton is called "distraction epiphysiolysis." This utilizes an external ring distractor to apply tension across an open growth plate, with external pins placed across the epiphysis and metaphysis. Its use is associated with risk of damage to the growth plate resulting in growth reduction [15] and therefore is often reserved

Table 2.1 Relative popularity of anatomic sites of distraction osteogenesis, based on the number of references obtained from a PubMed query (01/2016) using the search terms "distraction osteogenesis" and the "operative sites/indications"

Appendicular skeleton		Craniofacial skeleton	
Search term	# References	Search term	# References
Tibia	558	Mandible	1529
Femur	243	Maxilla	742
Radius	74	Alveolus	588
Metatarsal	58	Palate	476
Metacarpal	39	LeFort	420
Humerus	32	Craniosynostosis	287
Pelvis	9	Monobloc	80

for adolescents nearing closure of the growth plates. The membranous bones of the craniofacial skeleton lack growth plates, but during childhood individual bones may be distracted across interosteal suture lines. Palatal expansion is a well-known example of this trans-sutural approach. Recent studies suggest this approach may also be applied to the maxilla for midface advancement [16–18]. Without an osteotomy there is limited control of the bone using this method, and its value will require a future assessment of relapse and long-term outcomes.

2.3 Phases of Distraction Osteogenesis

Distraction osteogenesis may be divided into three dynamic or temporal phases: *latency*, *activation*, and *consolidation*. The period of delay following the osteotomy and prior to activation of the distraction device is known as the latency period. Short latency periods are generally associated with decreased volume of callus and inadequate osteogenesis, whereas long latency periods may lead to premature consolidation [4]. However, latency duration for the craniofacial skeleton (0–4 days) is by necessity much shorter than that for the appendicular skeleton (5–10 days), because of more rapid bone healing of the thin, membranous bones. Also, the majority of craniofacial distraction is performed in children, whose skeleton is actively growing, and who heal facial fracture rapidly at baseline. Some have even advocated for eliminating the latency phase in craniofacial applications. Slack et al. found that, although there are decreases in osteogenic activity at the cellular and molecular level when latency is eliminated, clinically there is no difference in the distracted mandible receiving a 48 h latency versus no latency period [19]. Other human [20] and animal models of mandibular distraction [21, 22] similarly found no clinical difference when the latency period was eliminated. Given these data and our clinical experience, a determination of appropriate latency period in craniofacial applications should be optimized to avoid premature consolidation. In neonates the latency period can be reduced to 1 day.

The *activation phase* follows latency. Its duration is determined by the clinical goal for bone production. Two variables during activation are the *rate* (or

distance) the device is advanced each day and the *rhythm* (or frequency) of device activation. In his canine tibial distraction model, Ilizarov found that an activation rate of 0.5 mm/day frequently resulted in premature consolidation, but 2.0 mm/day may result in nonunion [4]. McCarthy and the NYU group also reported that 1.0 mm/day had the best outcomes and that increased frequency of device activation resulted in fewer complications [23, 24]. From this many have cited a rate of 1.0 mm/day as the optimal rate for craniofacial device activation [21, 25, 26]. Mathematical [27] and computational [28] models seem to support this clinical finding. However, as with latency, neonates or young children with high healing proclivity may require faster distraction rates, up to 2.0 mm/day, to avoid premature consolidation [20].

Distraction *rhythm* refers to the frequency of activation. Using his canine tibial model, Ilizarov found increased quality and quantity in bone formed by distraction osteogenesis, when activating the distractor 60 times per day compared to only once per day, although both received a total of 1 mm advancement [4]. Histochemical analysis shows increased expression of osteoblastic markers (alkaline phosphatase and adenosine triphosphatase) in the tissues distracted with greater frequency [4]. A direct comparison between continuous and discontinuous distraction protocols further demonstrated improved vascularization and more rapid bone formation in the continuously activated protocol [29]. These findings have led to development of automated continuous distraction devices currently in preclinical testing stages [30]. It is important to note that the rhythm of distraction is not as important as the rate, and a rhythm of twice a day has become the accepted clinical model.

Following activation, the *consolidation phase* ensues during which time mineralization of the new osteoid matrix occurs. For membranous bones and prior to consolidation, it is possible to manipulate the distracted segment, a maneuver known as "molding the regenerate." Reports of this maneuver are largely limited to small case series, which demonstrate that in certain instances it may serve as a useful added step in the distraction protocol to help optimize the generated bone position, particularly to improve dental relationships [31–33]. Compared to the other stages of distraction osteogenesis, little research or controversy surrounds the length of this phase, and yet, this is the longest phase, especially for the patient. Upon completion of activation, the devices are kept in place until radiographic evidence of mineralization is present. In the craniofacial skeleton, the accepted period is at least twice the length of the activation phase, or approximately 8 weeks [34–36]. Inadequate time allowed for consolidation may lead to greater relapse of the regenerate.

2.4 Biology of Bone Formation, Fracture Healing, and Distraction Osteogenesis

Clinical use of distraction osteogenesis is essentially a form of bone tissue engineering. Tissue engineering requires three primary components: a progenitor or stem cell to produce the desired tissue, growth factors to provide the necessary inductive

signals to the progenitor cells, and a scaffold to guide appropriate three-dimensional configuration of the growing tissue. During distraction osteogenesis the bone-anchored distraction device provides the rigidity and necessary space that would normally be provided by a scaffold. Progenitor cells and growth factors are conveniently provided by the niche surrounding the distraction site. To the reconstructive surgeon hoping to generate new, vascularized bone, however, these cellular and molecular interactions may be a black box. Bone is unique among all tissues in the body, as it is the only tissue to heal or regenerate without scar formation and to regain its full premorbid strength and function. The complex molecular interactions of healing bone reflect how they formed during development [37, 38]. An understanding of the molecular biology and physiology of bone formation and fracture healing will provide insights into how bone is produced during distraction osteogenesis.

2.4.1 Pathways of Bone Development

During embryonic development, bone forms by one of two pathways: *endochondral* or *intramembranous* ossification (reviewed in [39]). The former requires a cartilaginous intermediate and is responsible for formation of the entire appendicular (limbs and pelvis) and much of the axial skeleton, including the ribs, scapulae, and skull base. *Endochondral bone* forms either from paraxial mesoderm (axial skeleton) or from lateral plate mesoderm, which contributes to the limb buds (appendicular skeleton). *Intramembranous ossification* does not involve a cartilaginous intermediate but instead relies on direct differentiation of mesenchymal precursor or neural crest cells into osteoblasts. It is the mechanism for development of most of the craniofacial skeleton. Intramembranous bones within the craniofacial skeleton (Fig. 2.3) are derived either from neural crest cells for the more cephalad structures and facial bones or from paraxial mesoderm for the more caudal structures and skull base [41]. Some of the caudal-most bones of the skull (occipital, ethmoid, petrous portion of the temporal, and portions of the sphenoid bones) develop by endochondral ossification.

Endochondral and intramembranous bone are first identified as clusters of undifferentiated cells known as mesenchymal condensations, which through an unknown mechanism coalesce in the areas of future skeletal development [39, 42]. Neural crest cells are derived from neuroectoderm of the developing neural tube, but undergo an epithelial-to-mesenchymal transition followed by delamination and ventral migration into craniofacial structures within the developing embryo. As with mesoderm-derived cells within mesenchymal condensations, neural crest cells similarly may lead to bone production via either intramembranous or endochondral ossification [43, 44], although the craniofacial skeleton is predominately formed from neural crest cells via intramembranous ossification (Fig. 2.4). The progression and differentiation of these cells are guided by signaling pathways, many of which are also relevant for fracture healing.

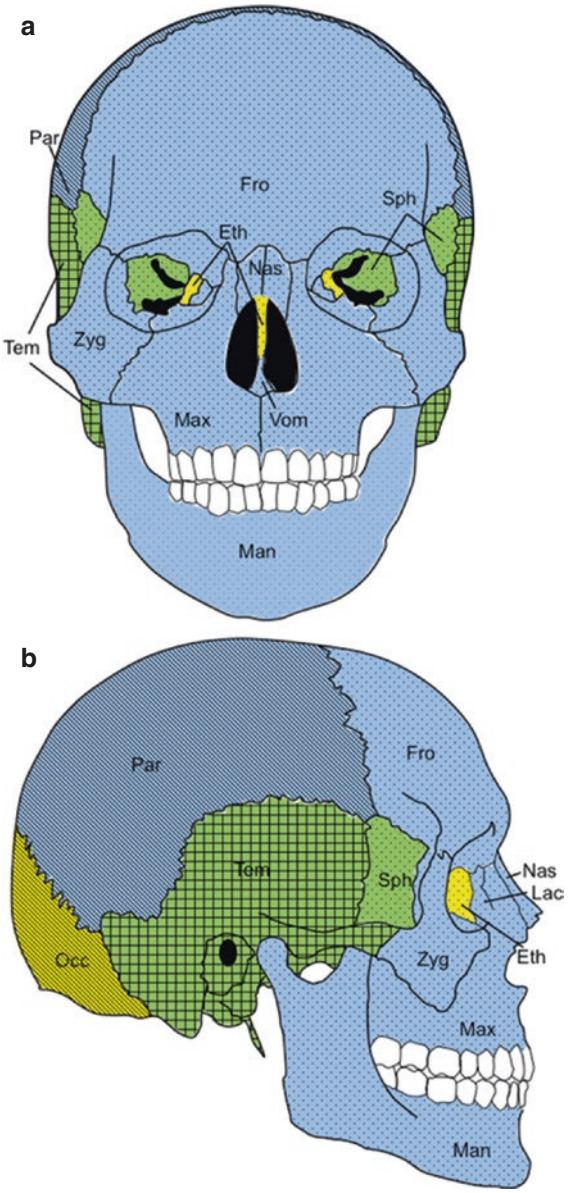

Fig. 2.3 Derivation of bones of the calvarium (adapted from [40], Craniofacial Embryogenetics and Development). (**a**, **b**) Two views of the human craniofacial skeleton, including (**a**) frontal and (**b**) lateral depicting both the cell source and the mechanism of bone formation. *Blue*—intramembranous ossification. *Yellow*—endochondral ossification. *Green*—both intramembranous and endochondral ossification. *Dotted*—neural crest cell derived. *Diagonal lines*—paraxial mesoderm derived. *Crosshatched*—both neural crest and paraxial mesoderm derived. *Eth* ethmoid, *Fro* frontal, *Lac* lacrimal, *Man* mandible, *Max* maxilla, *Nas* nasal, *Occ* occipital, *Par* parietal, *Sph* sphenoid, *Tem* temporal, *Vom* vomer, *Zyg* zygoma

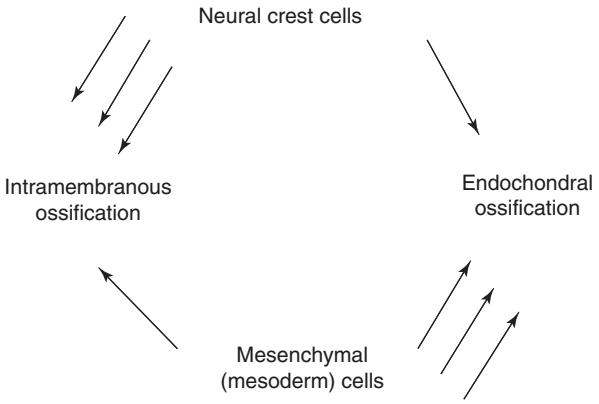

Fig. 2.4 Relative contributions of neural crest cells and paraxial mesoderm cells to the two types of bone within the craniofacial skeleton

Figure 2.5 depicts the possible fates of cells within the mesenchymal condensations. In the craniofacial skeleton, these cells may undergo intramembranous ossification, producing bone directly without a cartilaginous intermediate. In the remainder of the axial and appendicular skeleton, mesenchymal precursor cells give rise to an intermediate tissue, the immature cartilage. From immature cartilage two additional types of cartilage may develop: persistent and replacement cartilages. Persistent cartilage remains relatively avascular and eventually forms the cartilages of the nose, ear, intervertebral discs, and ribs. In contrast, replacement cartilage undergoes chondrocyte hypertrophy and vascularization allowing progression to endochondral ossification. During this process, chondrocytes enter a tightly controlled program of proliferation, pre-hypertrophy, hypertrophy, apoptosis, and replacement by osteoblasts [45].

Many of the signal transduction pathways regulating the progression of mesenchymal condensations to bone and cartilage are understood and are recapitulated in fracture healing. The pro-osteogenic factor runt-related transcription factor 2 (Runx2) is expressed among both pre-osteoblasts in mesenchymal condensations and later in immature cartilage [46]. Mice deficient in both alleles of Runx2 form no bone demonstrating its requirement for both intramembranous and endochondral bone formation [47–49]. Further, a mutation in one copy of Runx2 in humans leads to cleidocranial dysplasia which is marked by hypoplastic clavicles, supernumerary teeth, enlarged fontanelles, and eventual osteoporosis [48]. A similarly important pro-chondrogenic transcription factor, Sox9 [SRY (sex-determining region Y)-related HMG box gene 9], is essential for cartilage development. The absence of Sox9 in mice results in a complete absence of cartilage formation [50–52], and partial loss in humans leads to campomelic dysplasia [53–55], which is marked by craniofacial defects, bowing, and angulation of the long bones, and tracheobronchial hypoplasia which frequently leads to perinatal respiratory distress and lethality. Together Runx2 and Sox9 are master regulatory transcription factors for osteogenic and chondrogenic specification, respectively.

Sox9 promotes expression of essential cartilage-related collagen genes including Coll II [56], Coll IX [57], and Coll XI [58], which together help generate an

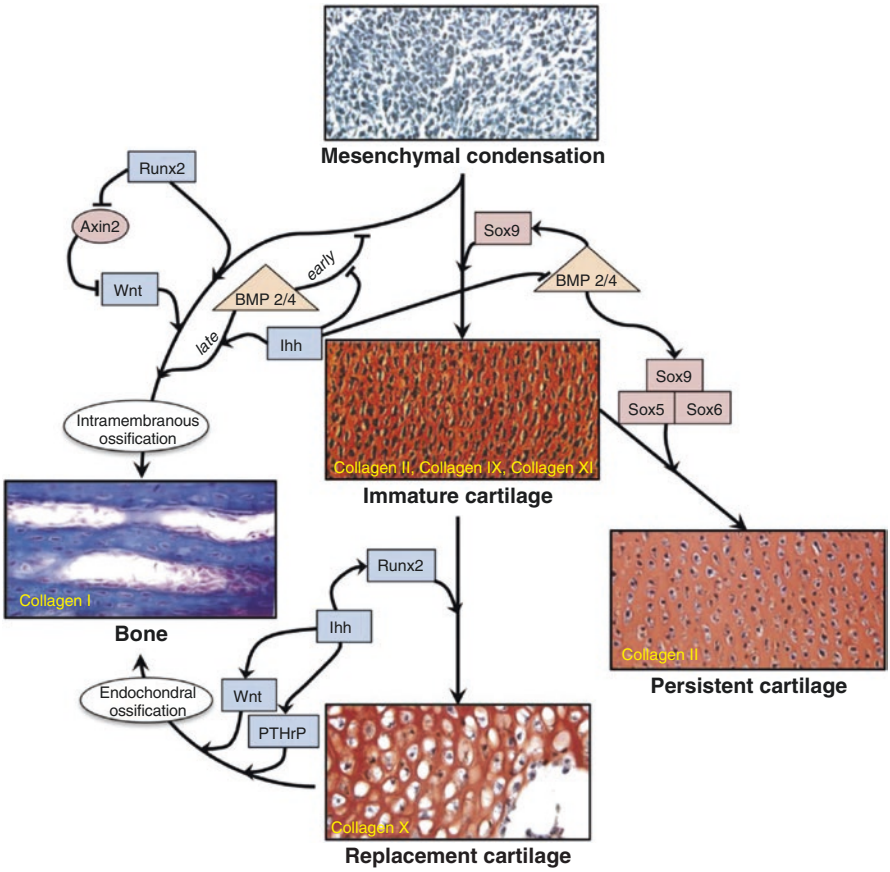

Fig. 2.5 Pathways for bone formation. Modified from Eames and Helms [37]

extracellular collagen matrix. Within immature cartilage chondrocytes rapidly divide and remain undifferentiated. Key factors in stimulating chondrocyte proliferation and Sox9 activity are bone morphogenetic proteins 2 (BMP-2) and BMP-4 [43]. This is perhaps counterintuitive because exogenous BMP-2 is clinically utilized as a powerful morphogen for bone formation. In contrast the transition of immature to replacement cartilage involves chondrocyte maturation through distinct pre-hypertrophic and hypertrophic stages, as well as vascular invasion and activation of bone markers. This requires additional signaling pathways, the most important of which is Hedgehog (reviewed in [59]).

The Hedgehog (Hg) gene is evolutionarily conserved, and mammalian homologues include Sonic (Shh), Desert (Dhh), and Indian (Ihh) hedgehogs. It is expressed by pre-hypertrophic chondrocytes within replacement cartilage and accelerates their hypertrophy and promotes osteoblast differentiation. Ihh does this by activating Runx2, which then activates Osterix (Osx) [60]; without either of these transcription factors, no bone can form. Ihh also decreases BMP-2 activity,

which leads to downregulation of Sox5, Sox6, Sox9, and Coll II [61]. Recent experiments performed in a bone organ culture system demonstrated that although BMP-2 has potent pro-osteoblast properties, Hh signaling is required; without the presence of Hh activity, BMP-2 promotes ectopic chondrogenesis within the perichondrium [62]. Ihh also stimulates expression of the hypertrophic cartilage marker, type-X collagen. Perhaps the best understood Ihh-mediated pathway in developing bone is that of parathyroid hormone-related peptide (PTHrP). Within growth plates of endochondral bone, Ihh and PTHrP participate in a feedback loop, regulating the rate of chondrocyte proliferation and differentiation into pre-hypertrophic and hypertrophic chondrocytes.

Development of calvarial bones by intramembranous ossification occurs as presumptive bone cells proliferate and migrate outward from mesenchymal condensations [63]. Instead of growth plates, intramembranous bone relies upon ossification centers that add bone in a radial fashion. Many of the pro-osteogenic molecular pathways essential for endochondral bone formation are essential for intramembranous bone formation, including Runx2, Wnt, Ihh, and BMP. A lack of BMP signaling within the cranial mesenchymal condensations is permissive for osteoblast formation, whereas at later stages BMP signaling is essential for neural crest-derived calvarial bone formation [41, 43]. Ihh also has an important role; it is expressed at the leading edge of growing cranial bones, promoting bone formation by BMP-2- and BMP-4-mediated direct osteogenic differentiation rather than proliferation [64]. Its loss results in significantly decreased calvarial bone formation [43]. Deletion of repressors of Hh signaling (Gli3 and Rab23) results in high Hh activity with associated increased ossification of calvarial bones and craniosynostosis [65, 66]. Runx2 is expressed within calvarial osteoblasts during the process and promotes osteogenesis. Loss of one allele of Runx2 is associated with delayed suture closure and persistent fontanels [67]. The pro-osteogenic effects of Runx2 in intramembranous bone are mediated through Wnt signaling. Activation of the Wnt pathway promotes specification of the osteogenic lineage and represses the chondrogenic lineage within calvarial mesenchyme [41]. TGF-β signaling is also important as it promotes calvarial osteocyte proliferation. Nearly all studies of intramembranous bone development examine the frontal or parietal bones, and relatively little is understood of the process within intramembranous bones of the facial skeleton [68].

2.4.2 Pathways of Appendicular Bone Fracture Healing

Fractures of bones of the appendicular skeleton heal by both intramembranous and endochondral ossification. Endochondral bone formation predominates, occurring outside the periosteum in mechanically unstable regions and immediately adjacent to the fracture site. Intramembranous bone formation occurs subperiosteally at the proximal and distal edges of the callus and forms hard callus [69]. Bridging of the hard callus across the fracture gap provides initial stabilization and leads to restoration of biomechanical function [70]. As endochondral ossification is the mechanism

of bone formation in the appendicular skeleton, it is also the mechanism primarily responsible for appendicular skeletal repair.

Four overlapping phases of fracture healing may be evident histologically (reviewed in [71]):

1) *Immediate inflammatory response*. This occurs over the initial 24–48 h post fracture and is marked by hematoma formation, hemostasis, inflammation, and recruitment of mesenchymal stem cells (MSCs).
2) *Cartilage formation with early endochondral ossification and periosteal response*. During this period mesenchymal stem cells differentiate into chondrocytes, which then produce a cartilaginous callus rich in collagen and proteoglycans [72, 73]. The soft, cartilaginous callus grows inversely proportional to the stability of the fracture and does so asymmetrically within the fracture. For example, femur fractures produce larger distal calluses and tibial fractures and larger proximal calluses, suggesting a recapitulation of bone development with the calluses forming nearest the growth plates [70, 74]. The soft callus growth peaks between 7 and 9 days following the fracture [73]. The periosteal response results in early intramembranous ossification and is associated with cell proliferation and early vascular ingrowth and neo-angiogenesis.
3) *Cartilage resorption and primary bone formation*. During this phase chondrocytes proliferate, mature, become hypertrophic, and increase synthesis of collagen, which accumulates within the extracellular matrix. As the chondrocytes then begin to undergo apoptosis, additional mesenchymal progenitor cells are recruited and differentiate into osteoblasts. This leads to callus mineralization, as osteoblasts use the soft callus as a template to deposit woven bone in place of the mineralized cartilage. This is initially manifest as a thin shell of bone around the periphery of the callus. Neo-angiogenesis also continues during this phase.
4) *Secondary bone formation and remodeling.* During this final phase, the bony callus grows and is reshaped by osteoclastic resorption and osteoblastic bone formation, resulting in regeneration of the original cortical and trabecular arrangement with a marrow-containing medullary cavity.

The molecular physiology of these four phases of fracture healing is well understood and shares many molecular similarities with endochondral bone development. A comprehensive description of these factors is beyond the scope of this chapter; however, an updated, concise summary is presented in Table 2.2. Of the many cytokines and growth factors involved, three groups have complex, well-defined overlapping roles during the four stages of bone healing: pro-inflammatory cytokines, TGF-β superfamily members (including the BMPs), and angiogenic factors. A number of other pathways are implicated in the healing process as their loss results in significant perturbations in the ability to heal, although their specific roles in the four phases of bone healing are not well defined. These include the Hedgehog [85] and Wnt signaling pathways ([86–88]; Minear 2010).

In the absence of rigid fixation, fracture healing of the appendicular skeleton occurs through formation of a cartilage scaffold, which is gradually replaced with

Table 2.2 Molecular pathway activation during endochondral bone fracture healing (adapted from [71])

Stage of fracture repair	Biologic process	Signaling molecule activation and proposed functions
Inflammation	Hematoma	IL-1, IL-6, and TNF-α release by circulating granulocytes and lymphocytes recruits inflammatory cells, enhances extracellular matrix synthesis, and stimulates angiogenesis [75]
	Inflammation and recruitment of progenitor cells	TGF-β, PDGF, and BMP-2 expression promote extracellular matrix formation and initial callus formation ([76], [77]). MMP-9 regulates the distribution of inflammatory cells [78]
Cartilage formation	Collagen deposition	Collagens type II and type III accumulate shortly after inflammation, produced by chondrocytes in the cartilaginous callus and periosteal osteoblasts
	Chondrogenesis and endochondral ossification	TGF-β2 and TGF-β3 stimulate chondrogenesis, corresponding with collagen type II synthesis [79]. BMP-2 promotes chondrocyte differentiation [80]. PTH also promotes cartilaginous and bony callus formation, whereas OPG prevents chondroclastogenesis by inhibiting RANKL
	Vascular ingrowth	MMP-9 promotes vascular invasion of hypertrophic cartilage, by promoting VEGF bioavailability [81]. VEGF directly stimulates angiogenesis and is maximally expressed when resorption is initiated [71]
Primary bone formation	Chondrocyte apoptosis and cartilage resorption	TNF-α stimulates mineralized chondrocyte apoptosis and cartilage resorption and helps recruit osteoprogenitor cells ([70, 82]; [83]). RANKL activity increases while OPG decreases, stimulating chondroclastogenesis
	Changes in collagen expression	Collagens type II and type III are removed as cartilage callus resorbs. Collagen type I accumulates as bony trabeculae develop. Collagen type X expression by hypertrophic chondrocytes provides a template for bone formation
	Mesenchymal cell differentiation to osteoblasts	Stimulated by BMP-2, BMP-6, and BMP-9 [84]
	Osteoblast recruitment and maturation	Stimulated by BMP-3, BMP-4, BMP-7, and BMP-8 ([79], [84])
	Neo-angiogenesis	VEGF and PDGF expression continue to promote angiogenesis
Secondary bone formation	Bone remodeling	TNF-α, IL-1, and RANKL activity promote bone remodeling by osteoclast remodeling of woven bone for lamellar bone formation

bone. This healing closely resembles the steps of embryonic endochondral ossification [38]. Mesenchymal precursors coalesce in the shape and location of the bone to be formed both for endochondral ossification and fracture healing. Both processes also involve mesenchymal cell proliferation and differentiation and hypertrophy along a cartilaginous or osteogenic pathway. An obvious difference between the processes is the presence of the inflammatory step in fracture healing that facilitates recruitment of the mesenchymal stem cells. However, once these cells are present, some of the same signaling pathways are involved including Ihh, VEGF, and MMP [38]. It is perhaps the preservation of many of these embryonic pathways that allow fractured bone to avoid forming scar, but to heal through a truly regenerative process.

2.4.3 Pathways of Craniofacial Skeletal Fracture Healing

An early rabbit mandible fracture model demonstrated that in the absence of rigid fixation, mandible fracture healing has some histologic similarities with long bone fractures [89]. Within 2 weeks of fracture, a large subperiosteal callus develops containing chondroid and immature osteoid. Within the subsequent 2 weeks, this callus is gradually replaced with trabecular bone and is completely bridged with new neovascular channels and Haversian systems. Paccione et al. similarly observed in their mouse mandible fracture model that the sequential presence of islands of rudimentary cartilage matrix formation, vascular ingrowth, osteoblast activation, mineralization, and lamellar bone formation resembled secondary bone endochondral bone healing [90]. They suggest that the contribution of a cartilage intermediate in their mandible fracture model (and that of others) was simply due to bony instability. Indeed the presence of instability in long bone fractures results in increased motion at the fracture site, which promotes cartilaginous callus formation during the primary bone healing phase.

Rigorous animal studies have not been performed to examine the histologic and molecular changes of facial bone fractures treated with rigid fixation. There are a number of reasons for this. The small size of rodent facial bones precludes plate fixation. Microplates were not available when bone healing studies were commonly performed. The lack of a straight marrow cavity precludes the use of intramedullary stabilization. Despite this, clinical experience provides overwhelming evidence that bones that develop by intramembranous ossification heal by the same mechanisms, and generally not through a cartilaginous intermediate. Skull fractures illustrate this principle. The scalp provides a tight soft tissue envelope to promote calvarial fracture reduction, while the convexity of the calvarium forms a sturdy keystone arch, which provides natural rigid fixation. Most of the bones of the facial skeleton similarly have a stabilizing periosteum and soft tissue envelope and are not subject to repeated forces. In contrast the mandible is subject to cyclic mechanical loading associated with mastication. However, with immobilization or rigid load-bearing or load-sharing fixation, the mandible heals by direct ossification.

Hasegawa et al. [91] provide experimental evidence opposing a role for chondrogenesis in membranous bone healing. They initially identified multipotent mesenchymal progenitor cells within fracture hematomas of long bones and demonstrated their ability to differentiate into osteocytes, adipocytes, and chondrocytes in vitro [92]. They subsequently cultured human *mandible* fracture hematoma cells and found that although these cells had a similar mesenchymal cell surface expression profile and had good osteogenic and adipogenic potential, they had a significantly reduced ability to differentiate into chondrocytes when compared to progenitors isolated from long bone fracture hematomas.

Compared with long bone fractures, our knowledge of the molecular physiology of healing craniofacial fractures is extremely sparse. Experiments in a rat model of mandible fracture healing implicate TGF-β superfamily members, including TGF-β1, BMP-2, BMP-4, and BMP-7, in osteoblast migration, differentiation, and proliferation [93, 94].

2.4.4 Physiologic Aspects of Distraction Osteogenesis on Bone Healing

Bones undergoing distraction osteogenesis share similar histologic characteristics of healing, regardless of whether they are within the craniofacial or appendicular skeleton [6, 95, 96]. However, there are significant histologic differences between distraction osteogenesis and fracture healing. The latency period of distraction resembles early fracture healing with hematoma formation and recruitment of inflammatory cells and mesenchymal stem cells [24, 71]. Endochondral bone formation may be observed during latency and early during distractor activation, although the endochondral bone is not found within the distraction gap but is limited to areas adjacent to the periosteum. Jazrawi [97] proposed that this observation suggests that the distraction environment may suppress cartilage development, but that a lack of device stability may be responsible for cartilage formation.

Rather than form a cartilaginous callus within the distraction gap, a physis-like structure of cells organizes into a fibrovascular bridge oriented in the direction of distraction called the *fibrous interzone*, or FIZ (see Fig. 2.6, [6, 34, 98]). The FIZ is rich in chondrocyte-like cells, fibroblasts, and oval cells, which are morphologically intermediates between fibroblasts and chondrocytes [6, 34, 98, 99]. As the distraction gap increases, the FIZ remains 4 mm thick, and at the conclusion of the process, the FIZ is the last region to ossify. Adjacent to the FIZ on either side is the *primary mineralization front* (PMF), which contains a high density of proliferating osteoblasts. These osteoblasts undergo primary mineralization in regions of newly formed capillaries and vascular sinuses, leading to formation of columns of bone resembling stalagmites and stalactites, known as the *zone of microcolumn formation* (MCF). When distraction ends, the PMF advances from each end toward the center, bridging the FIZ. Sequential mineralization of osteoid occurs during the activation and especially during the consolidation phase, starting within the surrounding MCF, which then proceeds to bridge the FIZ. During the consolidation period,

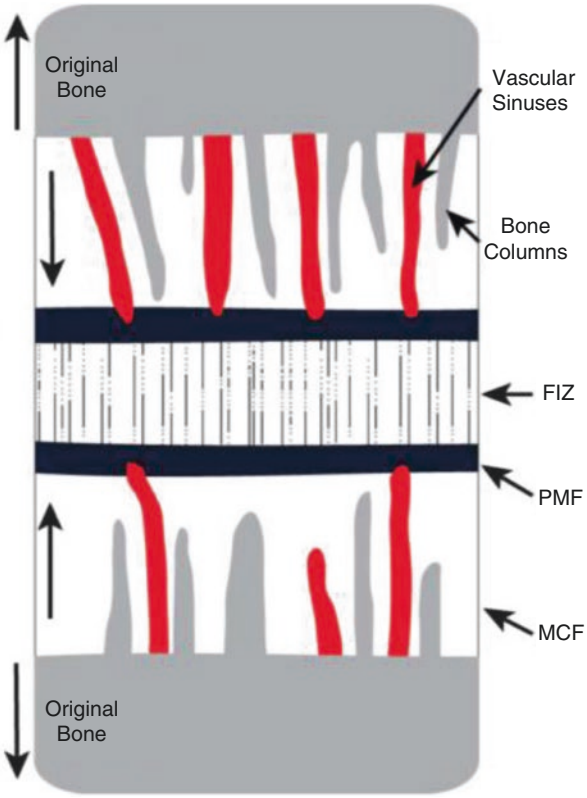

Fig. 2.6 Neo-physis of bone healing with distraction osteogenesis. See text for description. Reproduced, with permission, from [40]

mineralization of new bone is completed, and bony remodeling occurs resulting in formation of mature, lamellar bone with marrow.

The predominant mechanisms of bone formation within this niche are twofold. First, Yasui [100] observed that the FIZ of distracted rat femurs contained chondrocyte-appearing cells within a bony matrix, but without capillary ingrowth as is found in endochondral ossification. Similar to chondrocytes, these chondroid cells expressed type II collagen. However, they transition to type I collagen expression, suggestive of direct transformation of the chondrocyte-like cells into osteoblasts [101]. Yasui named this phenomenon "transchondroid bone formation" and proposed that it represents a new type of bone formation. However, both Yasui and others [3, 4, 6, 102] observed that the predominant mechanism of bone formation during distraction osteogenesis is intramembranous ossification, which may be distinguished from the other mechanisms by the histologic absence of cartilage and the expression of only type I collagen. At the ultrastructural level, disorganized bundles of type I collagen are found at the end of the latency period [103]. As activation begins, these bundles increase in size and become oriented in a plane parallel with the distraction force [6, 102, 104]. Osteoid is then deposited along the collagen bundles by osteoblasts located at corticotomy/osteotomy edges and within the distraction gap [104].

2.4.5 Molecular Aspects of Distraction Osteogenesis on Bone Healing

Distraction osteogenesis is initiated by an osteotomy. The molecular profile during the immediate post-osteotomy (latency) phase thus resembles that of fracture healing (Table 2.3). Pro-inflammatory cytokines IL-1 and IL-6 are upregulated in the

Table 2.3 Differential expression of signaling molecules during distraction osteogenesis (adapted from [71])

Signaling molecules	Latency		Activation		Consolidation	
	Early	Late	Early	Late	Early	Late
Cytokines						
IL-1[a]	+++					
IL-6[b]	+++		+++	+++		
TNF-α[a]						+++
RANKL/OPG ratio[a]		++	+++	+++	+	
TGF-β superfamily						
BMP-2[c]	++		+++	+++	+	
BMP-4[c]	++		+++	+++	+	
BMP-6[c] (at endochond)		+++	++	+		
TGF-β[c, d, j]	+	++	+++	+++	+	
Angiogenic factors						
VEGF-A[e]			+++	+++	+	
VEGF-B[e]			+	+	+	
VEGF-C[e]	++		+	+	+	
VEGF-D[e]		++	++	+		
Angiopoietin 1[e]		++	+	+		
Angiopoietin 2[e]			++	+		
HIF-1α[f]			+++	+++		
Other osteogenic factors						
FGF-2 (bFGF)[c]			++	++	+	+
IGF[c]			++	++		
Collagen I[d]	−−−	−−	−		+	+++
Osteocalcin[d, g, h]	−−−	−−	−	+	+	+
Osteopontin[i, g]			−/+++[i]	+++	?	?
Osteonectin[g, h]			−	+++	?	?

"+" indicates gene upregulation, whereas "−" indicates gene downregulation. Empty squares indicate a lack of data or lack of differential gene expression beyond baseline

[a]Ai-Aql et al. [71]

[b]IL-6—Cho et al. [105]

[c]BMP-2, BMP-4, BMP-6, BMP-7, GDF-5—Sato et al. [109], Nuntanaranont et al. [112] (BMP-2, BMP-4), Khanal et al. [113] (BMP-2, BMP-4)

[d]TGF-b, collagen I, osteocalcin—Mehrara and Longaker [114], Nuntanaranont et al. [112] (TGF-β)

[e]VEGFs and angiopoietin—Pacicca et al. [115]

[f]VEGFs and HIFα—Carvalho et al. [116]

[g]Opn, Oc, osteonectin, collagen I—Sato et al. [99]

[h]Osteonectin, osteocalcin—Meyer…Joos

[i]Opn—Perrien (2002) (varies by cell type)

[j]TGF-β superfamily—Choi et al. [117]

initial period, promoting extracellular matrix synthesis and inflammatory cell recruitment [71, 105]. Osteogenic and chondrogenic differentiation of these progenitors is similarly stimulated by early BMP-2 expression. A separate pro-inflammatory marker, TNF-α, is not expressed during latency, likely because its induction requires a greater traumatic insult than a simple osteotomy [105].

With device activation the molecular expression profile significantly deviates from that of fracture healing. IL-6 is upregulated a second time when activation begins and mechanical strain is applied to the callus. At this time its expression is high in osteoblasts, chondrocytes, and oval cells within the FIZ where tensile strains are the highest. IL-6 upregulation is thought to contribute to intramembranous ossification by enhancing osteogenic differentiation, and IL-6 has an anabolic effect on distraction osteogenesis and catabolic effect in fracture repair [105].

The TGF-β superfamily members are also upregulated during device activation. TGF-β was increased in distracted mandibles compared to those with non-distracted osteotomies [24], and a direct correlation between an increasing rate of mandibular distraction and TGF-β expression has been observed [106]. During activation TGF-β promotes osteoblast proliferation while suppressing their maturation, effectively delaying their differentiation and thus promoting new bone formation [107, 108]. BMP-2 and BMP-4 expression are both expressed immediately following the osteotomy, are downregulated, and then are highly reexpressed during device activation [109]. These BMPs are upregulated specifically within chondrogenic cells at the PMF and within oval cells within the FIZ, in response to the application of mechanical strain [109, 110]. They are maintained throughout activation, but then gradually disappear during consolidation, further implying a role in proliferation of cells required for completion of bone healing. Consistent with this, the addition of exogenous BMP-2 shortens treatment time during DO by accelerating bone formation during the consolidation phase [111]. In contrast to other factors, BMP-6 expression, limited to chondrocytes within the FIZ, begins during the latency phase and declines during the activation phase. BMP-6 downregulation occurs as the primary mode of ossification transitions from endochondral to intramembranous, reflecting its contributions to endochondral bone formation [109].

Two additional growth factors have been identified which are responsive to the increased mechanical strain found during device activation. Insulin-derived growth factor-1 (IGF-1) and fibroblast growth factor-2 (FGF-2, or basic FGF) are both highly expressed around the PMF and may promote osteoblast differentiation before subsequent downregulation during consolidation [22, 106].

As with fracture healing, osteoclastogenesis is necessary to help bone formed by distraction osteogenesis to remodel and form mature, lamellar bone. The RANKL/OPG system is thought to be the key regulator for balanced bone turnover [118]. As with fracture healing, a high RANKL/OPG expression ratio promotes osteoclastogenesis. The RANKL/OPG ratio increases late during latency and peaks within the consolidation phase, with the greatest turnover occurring at 3–4 weeks of consolidation [118, 119]. Activation of osteoclasts by TNF-α occurs throughout fracture healing; however, it is not expressed until later during consolidation, suggesting that RANKL/OPG plays the primary role for bone turnover and maturation [82].

Osteocalcin is expressed by mature osteoblasts and promotes mineralization. Its expression is significantly decreased compared with normal bone during the latency period. Osteocalcin levels gradually increase early during distraction, until reaching normal levels toward the end of consolidation [99, 114]. In contrast, osteocalcin in acutely lengthened mandibles does not significantly increase 6 weeks post distraction. This suggests deficiencies in osseous regeneration in acutely lengthened specimens are due to disturbances in mineralization/bone turnover in addition to decreased bone scaffold production.

2.4.6 Angiogenesis in Distraction Osteogenesis

Angiogenesis is an essential process for distraction osteogenesis. When angiogenesis is chemically inhibited, a lack of ossified bone and blood vessels occurs between the two cut ends of bone, with a resulting fibrous nonunion [120]. Mechanical distraction induces much greater angiogenic response than fracture healing [71]. Blood flow during activation increases up to 10 times the normal blood flow, as measured by quantitative technetium scintigraphy [34, 121]. Histologically, periosteal and endosteal vessels form columns alongside newly developing bone, toward the FIZ [79]. Within the FIZ capillaries are formed by both sinusoidal and transport capillary angiogenesis. During consolidation the periosteal and medullary vascular networks connect at the distraction site, including the FIZ [79]. Although new vessel formation begins during activation, maximal vessel volume increase occurs during consolidation, suggesting a link between angiogenesis and bone formation [122–124].

Among VEGF family members, only VEGF-A and neuropilin (a VEGF receptor) are significantly upregulated during the activation phase [116]. VEGF-D is upregulated briefly during the latency period but is diminished thereafter [116]. VEGF-A is expressed in maturing osteoblasts within the PMF and within osteoclasts in the MCF zone, directing angiogenesis in this region of the distraction gap [117]. Partial blockade of VEGF signaling in a tibial model of DO results in blockade of intramembranous ossification but allows for chondrogenesis, whereas complete VEGF blockade inhibits both osteogenesis and chondrogenesis [125]. The primary source of VEGF-A during DO is mesenchymal cells within the surrounding muscle. These blood vessels then synthesize morphogens (e.g., BMP-2) that promote bone formation in distracted bone [123]. An upstream activator of VEGF-A, HIF-1α, is significantly upregulated in bone undergoing distraction compared with fracture healing [115], suggesting that many of the downstream genes that are targets of HIF-1α (e.g., VEGF-A) play a major role in promoting new bone formation during DO. Deferoxamine enhancement of MDO is thought to be by upregulation of HIF-1α activity [126, 127]. Morgan [124] found that (1) the phase of activation is characterized primarily by arteriogenesis in surrounding muscle; (2) during consolidation, angiogenesis predominates in the intraosteal region; and (3) vessel formation proceeds from the surrounding muscle into the regenerate. Periods of intense osteogenesis are concurrent with those of angiogenesis.

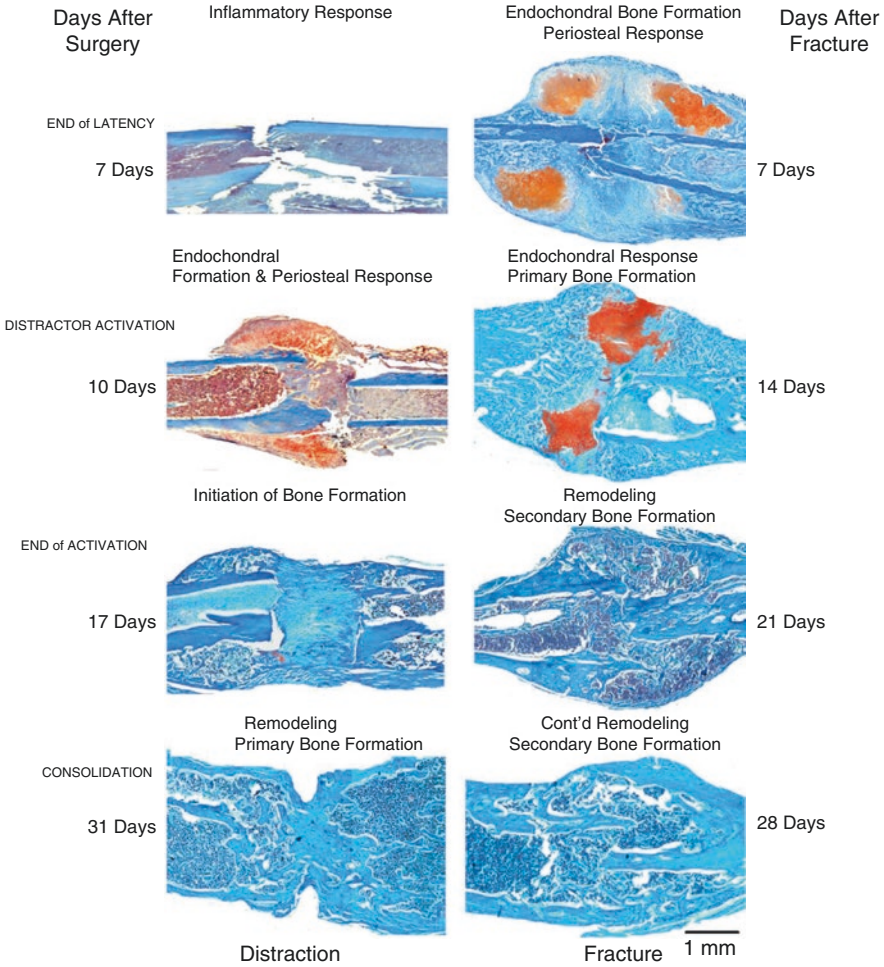

Fig. 2.7 Comparison of the progression of healing in fractures and distraction osteogenesis. Murine femur fracture calluses and tibial distraction gap tissues were prepared at the indicated time points, using Safranin-O/fast *green* staining. Cartilage is identified with *bright red* stain. The scale bar indicates 1 mm for all panels. Used with permission from [71]. The presence of cartilage during distractor activation may indicate some device instability

2.4.7 Contrasting Bone Formation by Fracture Healing and Distraction Osteogenesis

Distraction osteogenesis shares aspects of some of the physiologic pathways of fracture healing, but is clearly a distinct biologic process. This can be easily appreciated by comparing the two processes histologically (Fig. 2.7, [71]). Shortly after fracture of the appendicular skeleton, a robust cartilage callus forms outside the bone, stabilizing the fracture. In distraction osteogenesis, much less cartilage is

formed, and its presence is temporally restricted to the early periods after activation is initiated, after which it is rapidly resorbed. Distracted bone also has large amounts of unmineralized osteoid in the central region of the distraction gap, whereas the fracture callus of endochondral bone calcifies rapidly as it undergoes primary bone healing. Bragdon [122] speculates that the lack of cartilage formation during distraction is due to the population of precursor cells that reside within the endosteum. Endosteal cells are restricted to the osteogenic lineage, whereas the periosteum, which contributes to both fracture healing and distraction osteogenesis, has precursor cells capable of differentiating into both chondrocytes and osteoblasts [128].

Angiogenesis is critical for both fracture healing and distraction osteogenesis. VEGFs are expressed during both processes but have higher relative expression during fracture healing. VEGF receptor knockout studies showed that both angiogenesis and osteogenesis during distraction osteogenesis were dependent on activity of both VEGF receptors 1 and 2 [125]. Also, inhibition of VEGF in a fracture-healing model showed delayed healing and failure to progress from a cartilaginous to bony callus. In fracture healing, angiogenesis begins between days 7 and 14 as chondrogenic tissues undergo resorption [71]. However, during distraction osteogenesis, angiogenesis is initiated only after activation has begun and is thought to be driven by the distraction process rather than by signals elaborated from chondrocytes [71, 122]. The observation that the majority of new vessels occur within the medullary space of the distraction regenerate supports this theory [115, 125]. This is in contrast to fracture healing, wherein new vessel formation occurs within the external callus and is associated with the cartilage-to-bone transition.

In certain respects, distraction osteogenesis more closely resembles embryonic bone development than fracture healing. The rate of bone formation during distraction osteogenesis is 200–400 μM/day, which is 4–8× faster than the fastest physeal growth in adolescence, and is equivalent to that of the fetal femur [95, 96, 117]. There is also circumstantial evidence that pathways that are important for bone development are differentially regulated during distraction osteogenesis. Shibazaki reported increased PTHrP activity within distracted mandibular condyles [129]. Kasaai found significant increases in Wnt signaling factors in a mouse tibial distraction model [130]. Hedgehog signaling is also altered in a rabbit model of calvarial distraction [131]. However, there is not enough understanding of DO to determine whether it is a physiologic recapitulation of embryonic bone development. This is certainly an area for future study.

2.5 Biomechanics of Distraction Osteogenesis

Simply stated, biomechanics refers to the effects that mechanical forces have on biologic processes. The distraction process translates mechanical forces to a predictable biologic endpoint. At the distraction site, the mechanical factors that influence the environment include the applied tensile distractive forces, the rigidity of the fixation device, the amount of physiologic loading (muscle action), and the properties of the

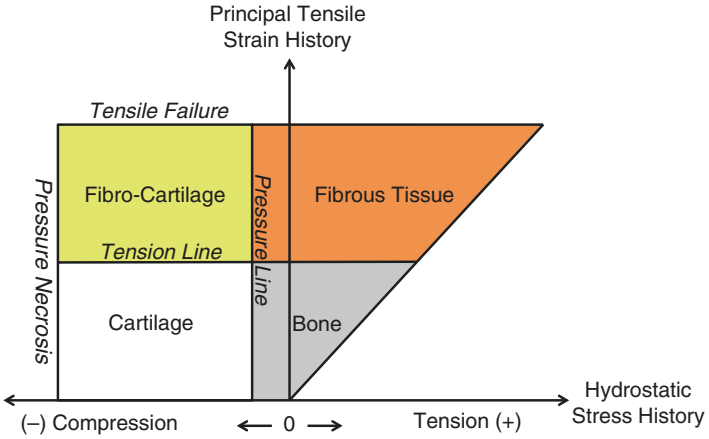

Fig. 2.8 Phase diagram of tissue differentiation concept relating mechanical loading history of multipotent mesenchymal tissue to skeletal tissue formation. Tensile failure line marks cutoff region beyond which failure of tissue occurs and new mesenchymal tissue forms in response to tissue trauma [134, 135]. Adapted with permission from [135]

surrounding soft tissues and regenerate. As distraction proceeds, one would expect tensile forces to increase. This has been confirmed in studies of human limb lengthening, with tensile forces increasing from 2.5 N/kg at initiation of activation and leveling off at 9.5 N/kg at completion [132]. This increase is likely caused by a combination of increasing resistance from the soft tissues and growing bony regenerate. During consolidation the ratio of force carried by the fixator versus the distracted limb can be measured. Increasing mineralization of the regenerate results in an increase in axial stiffness and a decrease in this ratio. For the appendicular skeleton, the regenerate's load-bearing capacity at the beginning of consolidation is 45% and, at least 4 months of consolidation, is typically required to improve to 90% [132, 133].

The type and intensity of the applied forces directly influence bone formation. Finite element modeling of mouse tibial fracture healing and distraction osteogenesis has led to characterization of these influences (Fig. 2.8, [134, 135]):

(1) Intermittent loading in regenerating bone heals by direct intramembranous bone formation in areas of low stress and strain.
(2) Low to moderate magnitudes of tensile strain and hydrostatic tensile stress also stimulate intramembranous ossification.
(3) Poor vascularity can promote chondrogenesis in an otherwise osteogenic environment.
(4) Hydrostatic compressive stresses stimulate chondrogenesis.
(5) High tensile strain stimulates production of fibrous tissue.
(6) Tensile strain with superimposed hydrostatic compressive stress stimulates development of fibrocartilage.
(7) Low shear stress favors cartilage and high shear stress results in fibrous tissue formation.

These principles have been validated in the craniofacial skeleton using a rat mandibular model of the latency and activation phases of distraction osteogenesis [135, 136]. Ultimately, for successful intramembranous bone formation by distraction osteogenesis, a low to moderate magnitude of tensile force (up to 13% reported by Loboa [135]) is required. Instability of the fixation device or shear stresses will favor endochondral bone formation.

2.6 Mechanotransduction and Mechanocoupling

Mechanotransduction is the translation of mechanical loading to cellular signal transduction pathway activation. Bone cells sense the applied tensile forces during distraction and transform these stimuli into biochemical signals into the cellular responses leading to appropriate changes in the architecture of the healing bone [137]. Mechanotransduction consists of the following steps: (1) mechanocoupling, (2) signal transmission, and (3) the effector cell response [138]. Mechanocoupling is the initial detection of a mechanical force with an associated signal pathway activation. The cell within bone responsible for initially sensing and responding to these forces is thought to be the osteocyte [139]. This is because osteocytes are regularly distributed throughout cortical and trabecular bone, because they are connected and communicate through long cellular processes and because they are unlikely to be effector cells due to being trapped within bone [140, 141]. The protein or structure within osteocytes responsible for mechanocoupling during distraction osteogenesis has not been identified, but there are a number of candidates [139]. The cells' cytoskeletons may directly sense changes in cell shape associated with the tensile forces. This "substrate deformation" may act directly on the actin cytoskeletons of the long osteocyte processes or the cell body itself [142, 143]. Alternatively, changes in the lacunocanalicular flow between osteocytes may provide the signals [144]. This may involve activation of stretch- or voltage-activated ion channels, G-protein-coupled receptors, and nodal cilia [140, 145]. Likely, multiple of these mechanisms are involved in sensing the distraction tensile forces.

Following mechanocoupling of the tensile force to the osteocyte, a series of secondary biochemical signaling events occurs, including changes in gene expression, protein and lipid modifications, protein degradation, alteration in cell shape/size, and the release of secreted factors. Collectively these events allow signal propagation within the osteocyte and activation of effector cells, namely, osteoblasts and osteoclasts. A number of signaling pathways have been identified that, when inactivated, inhibit the response of bone to a loading stress, including cyclooxygenase-2/prostaglandins [146], Wnt/LRP-5/β-catenin [147], IGF-1 [148, 149], and nitric oxide [150] pathways. Effector cell responses are manifest in protein expression by osteoblasts and osteoclasts, as new bone is produced. For example, alkaline phosphatase, type I collagen, osteopontin, osteocalcin, Runx2, and Osterix are all upregulated in response to mechanical loading of bone. Specific to distraction osteogenesis, Table 2.3 listed many of the other factors involved in both the signal transmission and effector cell phases of mechanotransduction.

2.7 Advances of Distraction Osteogenesis in the Craniofacial Skeleton

Because this chapter has introduced the foundation of bone healing physiology and biomechanics, a significant focus has been placed upon the historic development of distraction osteogenesis within long bones. Limb lengthening will continue to be a useful tool to the orthopedist, but the frontiers in distraction osteogenesis seem to lie in craniofacial applications (as supported by Table 2.1). As proposed by Dr. McCarthy in his prologue to the initial edition of this text, the craniofacial skeleton is more suited to surgical distraction than the long bones for the following reasons: superior blood supply, easier surgical accessibility, decreased associated pain, shorter required distraction/consolidation period, greater ease of measuring outcomes (dental measurements and cephalograms), and relatively lesser morbidity with wide, subperiosteal dissection.

This speculation has been borne out by the expanded clinical use of distraction osteogenesis within the craniofacial skeleton and the development of a larger literature. Initially described to improve mandibular asymmetry in patients with craniofacial microsomia, craniofacial distraction osteogenesis today is more commonly used to correct severe functional deficits. For example, distraction of the mandible is frequently used to correct tongue-based airway obstruction in neonates with micrognathia or in adults with severe obstructive sleep apnea. Midface distraction has supplanted the traditional or acute advancement technique, especially in the growing child. Posterior cranial vault distraction is used to delay the need for major cranial remodeling by reducing high intracranial pressure in patients with syndromic craniosynostosis, until a time that major surgery can more safely be performed. Distraction osteogenesis will continue to be an important tool in the craniofacial surgeon's armamentarium for treatment of difficult orthognathic or reconstructive cases.

The miniaturization and internalization of external distraction devices have been particularly beneficial to craniofacial applications. Frequently used bilaterally, internal or semi-buried devices have less failure, increased rigidity and stability, and greater convenience for patients and their families compared to the large, conspicuous external devices. External devices are thought to provide greater control over midface distraction vectors and permit molding of the generate during the activation and consolidation phases. Internal devices require a second operation for device removal and require greater periosteal undermining for placement.

Another advance in craniofacial distraction is the growing potential for adjuvant therapies to accelerate and improve the process. Preclinical animal models demonstrate improved bone formation during mandibular distraction osteogenesis (reviewed in [151]) with the addition of growth factors (BMP-2, BMP-4, BMP-7, IGF-1, VEGF, growth hormone, adiponectin, erythropoietin), osteoclast-suppressive medications (alendronate, zoledronic acid), mesenchymal stem cells, hyperbaric oxygen, and a number of mechanical stimuli (low-intensity shock wave therapy, low-intensity pulsed ultrasound). These models are predicated upon an understanding of the basic biomechanics and molecular physiology of bone

healing and distraction osteogenesis. These basic principles provide a vast opportunity for both optimizing distraction osteogenesis and reducing the length of the clinical therapeutic process.

Buchman at the University of Michigan developed a high-throughput, reproducible model of rat mandibular distraction (REFs), permitting investigation of adjuvants and new applications for distraction osteogenesis [152]. The pro-angiogenic factor HIF-1α is one such factor [116]. Given its significant upregulation during the activation phase of distraction osteogenesis, it was hypothesized that increasing HIF-1α activity by deferoxamine administration would enhance bone formation during distraction osteogenesis. They demonstrated that deferoxamine increased HIF-1α levels in their model, resulting in enhanced vascular formation [126], more rapid consolidation of the regenerate (Donneys 2013), and greater bone production [127, 153]. Buchman has also examined the effects of radiation on distraction osteogenesis. Osteoradionecrosis of the mandible, a difficult reconstructive challenge, frequently requires autologous bone flaps. It was found that, in response to radiation, bone produced by distraction had significantly reduced osteocyte numbers, decreased bone mineralization, decreased vascularity, and a lower breaking load compared to control hemi-mandibles [154–157]. They also demonstrated that concomitant treatment with a number of factors had a protective effect from radiation damage, including parathyroid hormone [158, 159], amifostine [153, 160], and stem cells [154].

Pearls and Pitfalls

- Classification methods of distraction osteogenesis include treatment goal, distraction device type, anatomic location, and operative approach.
- Phases of distraction osteogenesis include latency, activation, and consolidation. Variables during the activation phase include the rate and rhythm of device activation.
- An understanding of embryonic bone formation and fracture healing helps one understand the physiology and biology of distraction osteogenesis.
- Bone generated by distraction osteogenesis does so predominately by intramembranous ossification, regardless of anatomic location. This is also the same mechanism of bone formation of developing craniofacial bone and fracture healing.
- The type and intensity of applied forces during distraction influence the types of tissue created.

References

1. Codivilla A. On the means of lengthening, in the lower limbs, the muscles and tissues which are shortened through deformity. Am J Orthop Surg. 1905;(4):353–69. http://jbjs.org/content/s2-2/4/353.abstract
2. Jordan CJ, Goldstein RY, Mclaurin TM, Grant A. The evolution of the Ilizarov technique: part 1: the history of limb lengthening. Bull NYU Hosp Jt Dis. 2013;71(1):89–95.
3. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part II. Clin Othop Relat Res. 1989a;(239):263–85.
4. Ilizarov GA. The tension-stress effect on the genesis and growth of tisues. Part I. Clin Orthop Relat Res. 1989b;(239):263–85.
5. Snyder CC, Levine GA, Swanson HM, Browne EZ. Mandibular lengthening by gradual distraction. Plast Reconstr Surg. 1973;51(5):506–8.
6. Karp NS, McCarthy JG, Schreiber JS, Sissons HA, Thorne CHM. Membranous bone lengthening: a serial histological study. Ann Plast Surg. 1992;29(1):2–7.
7. Karp NS, Thorne CHM, McCarthy JG, Sissons HA. Bone lengthening in the craniofacial skeleton. Ann Plast Surg. 1990;24(3):231–7.
8. McCarthy JG, Schreiber JS, Karp NS, Thorne CHM, Grayson BH. Lengthening the human mandible by gradual distraction. Plast Reconstr Surg. 1992;89(1):1–8.
9. Rachmiel A, Potparic Z, Jackson IT, Sugihara T, Clayman L, Topf JS, Forté RA. Midface advancement by gradual distraction. Br J Plast Surg. 1993;46(3):201–7. http://www.ncbi.nlm.nih.gov/pubmed/8490698
10. Staffenberg DA, Wood RJ, McCarthy JG, Grayson BH, Glasberg SB. Midface distraction advancement in the canine without osteotomies. Ann Plast Surg. 1995;34(5):512–7.
11. Glat PM, Staffenberg DA, Karp NS, Holliday RA, Steiner G, McCarthy JG. Multidimensional distraction osteogenesis: the canine zygoma. Plast Reconstr Surg. 1994;94(6):753–8.
12. Bouletreau PJ, Warren SM, Paccione MF, Spector JA, McCarthy JG, Longaker MT. Transport distraction osteogenesis: a new method to heal adult calvarial defects. Plast Reconstr Surg. 2002b;109(3):1074–84. http://www.ncbi.nlm.nih.gov/pubmed/11884839
13. Losken HW, Mooney MP, Zoldos J, Tschakaloff A, Burrows AM, Smith TD, et al. Internal calvarial bone distraction in rabbits with delayed-onset coronal suture synostosis. Plast Reconstr Surg. 1998;102(4):1109–19; discussion 1120–1. http://www.ncbi.nlm.nih.gov/pubmed/9734430
14. Guichet J-M, Deromedis B, Donnan LT, Peretti G, Lascombes P, Bado F. Gradual femoral lengthening with the Albizzia intramedullary nail. J Bone Joint Surg Am. 2003;85-A(5):838–48. http://www.ncbi.nlm.nih.gov/pubmed/12728034
15. Synder M, Niedzielski K, Borowski A. Complication, difficulties and problems in the application of distraction epiphysiolysis. Ortop Traumatol Rehabil. 2002;4(4):464–8. http://www.ncbi.nlm.nih.gov/pubmed/17679880
16. Coeugniet E, Dhellemmes P, Vinchon M, Wolber A, Pellerin P. Midfacial distraction without osteotomy using a transfacial pin and external devices. J Craniofac Surg. 2012;23(1):184–9. doi:10.1097/SCS.0b013e3182418f80.
17. Tong H, Gao F, Yin J, Shi Z, Song T, Li H, et al. Three-dimensional quantitative evaluation of midfacial skeletal changes after trans-sutural distraction osteogenesis for midfacial hypoplasia in growing patients with cleft lip and palate. J Craniomaxillofac Surg. 2015a;43(9):1749–57. doi:10.1016/j.jcms.2015.08.027.
18. Tong H, Wang X, Song T, Gao F, Yin J, Li H, et al. Trans-sutural distraction osteogenesis for midfacial hypoplasia in growing patients with cleft lip and palate: clinical outcomes and analysis of skeletal changes. Plast Reconstr Surg. 2015b;136(1):144–55. doi:10.1097/PRS.0000000000001375.

19. Slack GC, Fan KL, Tabit C, Andrews B, Hindin DI, Kawamoto HK, Bradley JP. Necessity of latency period in craniofacial distraction: investigations with in vitro microdistractor and clinical outcomes. J Plast Reconstr Aesthet Surg. 2015;68(9):1206–14. doi:10.1016/j.bjps.2015.04.012.
20. Hollier LH, Higuera S, Stal S, Taylor TD. Distraction rate and latency: factors in the outcome of pediatric mandibular distraction. Plast Reconstr Surg. 2006;117(7):2333–6. doi:10.1097/01.prs.0000219354.16549.c9.
21. Glowacki J, Shusterman EM, Troulis M, Holmes R, Perrott D, Kaban LB. Distraction osteogenesis of the porcine mandible: histomorphometric evaluation of bone. Plast Reconstr Surg. 2004;113(2):566–73. doi:10.1097/01.PRS.0000101061.99577.09.
22. Tavakoli K, Yu Y, Shahidi S, Bonar F, Walsh WR, Poole MD. Expression of growth factors in the mandibular distraction zone: a sheep study. Br J Plast Surg. 1999;52(6):434–9. doi:10.1054/bjps.1999.3157.
23. McCarthy JG, Stelnicki EJ, Grayson BH. Distraction osteogenesis of the mandible: a ten-year experience. Semin Orthod. 1999;5(1):3–8.
24. McCarthy JG, Stelnicki EJ, Mehrara BJ, Longaker MT. Distraction osteogenesis of the craniofacial skeleton. Plast Reconstr Surg. 2001;107(7):1812–27.
25. Farhadieh RD, Gianoutsos MP, Dickinson R, Walsh WR. Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. Plast Reconstr Surg. 2000;105(3):889–95. doi:10.1097/00006534-200003000-00010.
26. Mofid MM, Manson PN, Robertson BC, Tufaro AP, Elias JJ, Vander Kolk CA. Craniofacial distraction osteogenesis: a review of 3278 cases. Plast Reconstr Surg. 2001;108(5):1103–14; discussion 1115–7. http://www.ncbi.nlm.nih.gov/pubmed/11604605
27. Schendel SA, Heegaard JH. A mathematical model for mandibular distraction osteogenesis. J Craniofac Surg. 1996;7(6):465–8.
28. Boccaccio A, Pappalettere C, Kelly DJ. The influence of expansion rates on mandibular distraction osteogenesis: a computational analysis. Ann Biomed Eng. 2007;35(11):1940–60. doi:10.1007/s10439-007-9367-x.
29. Djasim UM, Mathot BJ, Wolvius EB, van Neck JW, van der Wal KGH. Histomorphometric comparison between continuous and discontinuous distraction osteogenesis. J Craniomaxillofac Surg. 2009;37(7):398–404. doi:10.1016/j.jcms.2009.03.006.
30. Peacock ZS, Tricomi ÃBJ, Faquin WC, Magill JC, Murphy BA, Kaban LB, Troulis MJ. Bilateral continuous automated distraction osteogenesis: proof of principle. J Craniofac Surg. 2015;26(8):2320–4. doi:10.1097/SCS.0000000000001996.
31. Luchs JS, Stelnicki EJ, Rowe NM, Naijher NS, Grayson BH, McCarthy JG. Molding of the regenerate in mandibular distraction: part 1: laboratory study. J Craniofac Surg. 2002;13(2):205–11. doi:10.1097/00001665-200203000-00004.
32. McCarthy JG, Hopper RA, Hollier LH, Peltomaki T, Katzen T, Grayson BH. Molding of the regenerate in mandibular distraction: clinical experience. Plast Reconstr Surg. 2003;112(5):1239–46. doi:10.1097/01.PRS.0000080726.50460.3E.
33. Pensler JM, Goldberg DP, Lindell B, Carroll NC. Skeletal distraction of the hypoplastic mandible. Ann Plast Surg. 1995;134(2):130–6.
34. Aronson J. Experimental and clinical experience with distraction osteogenesis. Cleft Palate Craniofac J. 1994b;31(6):473–81. doi:10.1597/1545-1569(1994)031<0473:EACEWD>2.3.CO;2.
35. Hopper RA, Altug AT, Grayson BH, Barillas I, Sato Y, Cutting CB, McCarthy JG. Cephalometric analysis of the consolidation phase following bilateral pediatric mandibular distraction. Cleft Palate Craniofac J. 2003;40(3):233–40. doi:10.1597/1545-1569(2003)040<0233:CAOTCP>2.0.CO;2.
36. Polley JW, Figueroa AA. Rigid external distraction: its application in cleft maxillary deformities. Plast Reconstr Surg. 1998;102(5):1360–72.
37. Eames BF, De la Fuente L, Helms J a. Molecular ontogeny of the skeleton. Birth Defects Res C Embryo Today Rev. 2003;69(2):93–101. doi:10.1002/bdrc.10016.

38. Ferguson C, Alpern E, Miclau T, Helms JA. Does adult fracture repair recapitulate embryonic skeletal formation? Mech Dev. 1999;87(1–2):57–66. doi:10.1016/S0925-4773(99)00142-2.
39. Karaplis A. Embryonic Development of Bone and the Molecular Regulation of Intramembranous and Endochondral Bone Formation. In: Bilezikian J, Raisz L, Martin TJ, Editors. Principles of Bone Biology Vol. 1. 2008, ISBN: 9780123738844.
40. Percival CJ, Richtsmeier JT. Angiogenesis and intramembranous osteogenesis. Dev Dyn. 2013;242(8):909–22. doi:10.1016/j.biotechadv.2011.08.021.Secreted.
41. Ishii M, Sun J, Ting M-C, Maxson RE. The development of the calvarial bones and sutures and the pathophysiology of craniosynostosis. Curr Top Dev Biol. 2015;115:131–56. doi:10.1016/bs.ctdb.2015.07.004.
42. Eames BF, Helms JA. Conserved molecular program regulating cranial and appendicular skeletogenesis. Dev Dyn. 2004;231(1):4–13. doi:10.1002/dvdy.20134.
43. Abzhanov A, Rodda SJ, McMahon AP, Tabin CJ. Regulation of skeletogenic differentiation in cranial dermal bone. Development. 2007;134(17):3133–44. doi:10.1242/dev.002709.
44. Bhatt S, Diaz R, Trainor PA, Wu DK, Kelley MW, Tam PL, et al. Signals and switches in mammalian neural crest cell differentiation signals and switches in mammalian neural crest cell differentiation. Cold Spring Harb Perspect Biol. 2013;5:a008326. doi:10.1101/cshperspect.a008326.
45. Thorogood PV, Hinchliffe JR. An analysis of the condensation process during chondrogenesis in the embryonic chick hind limb. J Embryol Exp Morphol. 1975;33(3):581–606.
46. Ducy P, Karsenty G. Genetic control of cell differentiation in the skeleton. Curr Opin Cell Biol. 1998;10(5):614–9. http://www.ncbi.nlm.nih.gov/pubmed/9818172
47. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell. 1997;89(5):755–64. doi:10.1016/S0092-8674(00)80258-5.
48. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, et al. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell. 1997;89(5):773–9. http://www.ncbi.nlm.nih.gov/pubmed/9182765
49. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, et al. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell. 1997;89(5):765–71. http://www.ncbi.nlm.nih.gov/pubmed/9182764
50. Akiyama H, Chaboissier MC, Martin JF, Schedl A, De Crombrugghe B. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes Dev. 2002;16(21):2813–28. doi:10.1101/gad.1017802.
51. Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B. Sox9 is required for cartilage formation. Nat Genet. 1999;22(1):85–9. doi:10.1038/8792.
52. Yan YL, Miller CT, Nissen RM, Singer A, Liu D, Kirn A, et al. A zebrafish sox9 gene required for cartilage morphogenesis. Development. 2002;129(21):5065–79. http://www.ncbi.nlm.nih.gov/pubmed/12397114\n; http://dev.biologists.org/content/129/21/5065.full.pdf
53. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanović M, et al. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature. 1994;372(6506):525–30. doi:10.1038/372525a0.
54. Kwok C, Weller PA, Guioli S, Foster JW, Mansour S, Zuffardi O, et al. Mutations in SOX9, the gene responsible for campomelic dysplasia and autosomal sex reversal. Am J Hum Genet. 1995;57(5):1028–36.
55. Wagner T, Wirth J, Meyer J, Zabel B, Held M, Zimmer J, et al. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell. 1994;79(6):1111–20. http://www.ncbi.nlm.nih.gov/pubmed/8001137
56. Lefebvre V, Huang W, Harley VR, Goodfellow PN, de Crombrugghe B. SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1(II) collagen gene. Mol Cell Biol. 1997;17(4):2336–46. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=232082&tool=pmcentrez&rendertype=abstract

57. Zhang P, Jimenez SA, Stokes DG. Regulation of human COL9A1 gene expression. Activation of the proximal promoter region by SOX9. J Biol Chem. 2003;278(1):117–23. doi:10.1074/jbc.M208049200.
58. Liu Y, Li H, Tanaka K, Tsumaki N, Yamada Y. Identification of an enhancer sequence within the first intron required for cartilage-specific transcription of the α2(XI) collagen gene. J Biol Chem. 2000;275(17):12712–8. doi:10.1074/jbc.275.17.12712.
59. Yang J, Andre P, Ye L, Yang Y-Z. The Hedgehog signalling pathway in bone formation. Int J Oral Sci. 2015;14:73–9. doi:10.1038/ijos.2015.14.
60. Mak KK, Chen M-H, Day TF, Chuang P-T, Yang Y. Wnt/beta-catenin signaling interacts differentially with Ihh signaling in controlling endochondral bone and synovial joint formation. Development. 2006;133(18):3695–707. doi:10.1242/dev.02546.
61. Long F, Chung U, Ohba S, McMahon J, Kronenberg HM, McMahon AP. Ihh signaling is directly required for the osteoblast lineage in the endochondral skeleton. Development. 2004;131(6):1309–18. doi:10.1242/dev.01006.
62. Hojo H, Ohba S, Taniguchi K, Shirai M, Yano F, Saito T, et al. Hedgehog-Gli activators direct osteo-chondrogenic function of bone morphogenetic protein toward osteogenesis in the perichondrium. J Biol Chem. 2013;288(14):9924–32. doi:10.1074/jbc.M112.409342.
63. Yoshida T, Vivatbutsiri P, Morriss-Kay G, Saga Y, Iseki S. Cell lineage in mammalian craniofacial mesenchyme. Mech Dev. 2008;125(9–10):797–808. doi:10.1016/j.mod.2008.06.007.
64. Lenton K, James AW, Manu A, Brugmann SA, Birker D, Nelson ER, et al. Indian hedgehog positively regulates calvarial ossification and modulates bone morphogenetic protein signaling. Genesis. 2011;49(10):784–96. doi:10.1002/dvg.20768.
65. Jenkins D, Seelow D, Jehee F, Perlyn C, Alonso L, Bueno D, et al. RAB23 mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity. Am J Hum Genet. 2007;80(6):1162–70.
66. Rice DPC, Connor EC, Veltmaat JM, Lana-Elola E, Veistinen L, Tanimoto Y, et al. Gli3Xt-J/Xt-J mice exhibit lambdoid suture craniosynostosis which results from altered osteoprogenitor proliferation and differentiation. Hum Mol Genet. 2010;19(17):3457–67. doi:10.1093/hmg/ddq258.
67. Otto F, Kanegane H, Mundlos S. Mutations in the RUNX2 gene in patients with cleidocranial dysplasia. Hum Mutat. 2002;19(3):209–16. doi:10.1002/humu.10043.
68. Sperber G, Sperber SM, Guttmann GD. Craniofacial embryogenetics and development. 2nd ed. Shelton: PMPH; 2010.
69. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury. 2005;36(12):1392–404. doi:10.1016/j.injury.2005.07.019.
70. Gerstenfeld LC, Alkhiary YM, Krall EA, Nicholls FH, Stapleton SN, Fitch JL, et al. Three-dimensional reconstruction of fracture callus morphogenesis. J Histochem Cytochem. 2006;54(11):1215–28. doi:10.1369/jhc.6A6959.2006.
71. Ai-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC, Einhorn TA. Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. J Dent Res. 2008;87(2):107–18. doi:10.1177/154405910808700215.
72. Hankenson K, Zimmermann G, Marcucio RS. Biologic perspectives of delayed fracture healing. Injury. 2014;45(Suppl 2):S8–S15. doi:10.1002/jcp.24872.The.
73. Marsell R, Einhorn TA. The biology of fracture healing. Injury. 2011;42(6):551–5. doi:10.1016/j.injury.2011.03.031.THE.
74. Morgan EF, Mason ZD, Chien KB, Pfeiffer AJ, George L, Einhorn TA, Gerstenfeld LC. Micro-computed tomography assessment of fracture healing: relationships among callus structure, composition, and mechanical function. Bone. 2009;44(2):335–44. doi:10.1016/j.bone.2008.10.039.Micro-Computed.
75. Kon T, Cho TJ, Aizawa T, Yamazaki M, Nooh N, Graves D, et al. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. J Bone Miner Res. 2001;16(6):1004–14. doi:10.1359/jbmr.2001.16.6.1004.

76. Sandberg MM, Hannu TA, Vuorio EI. Gene expression during bone repair. Clin Orthop Relat Res. 1993;289:292–312.
77. Bostrom M. Expression of bone morphogenetic proteins in fracture healing. Clin Orthop Relat Res. 1998;(355 Suppl):S116–23. http://www.ncbi.nlm.nih.gov/pubmed/9917632
78. Wang X, Yu YY, Lieu S, Yang F, Lang J, Lu C, et al. MMP9 regulates the cellular response to inflammation after skeletal injury. Bone. 2013;52(1):111–9. doi:10.1016/j.bone.2012.09.018.MMP9.
79. Cho T-J, Gerstenfeld LC, Einhorn T a. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J Bone Miner Res. 2002;17(3):513–20. doi:10.1359/jbmr.2002.17.3.513.
80. Wang Q, Huang C, Xue M, Zhang. Expression of endogenous BMP-2 in periosteal progenitor cells is essential for bone healing. Bone. 2011;48(3):524–32. doi:10.1016/j.bone.2010.10.178.Expression.
81. Colnot C, Thompson Z, Miclau T, Werb Z, Helms JA. Altered fracture repair in the absence of MMP9. Development. 2003;130(17):4123–33. doi:10.1242/dev.00559.
82. Gerstenfeld LC, Cho TJ, Kon T, Aizawa T, Tsay A, Fitch J, et al. Impaired fracture healing in the absence of TNF-alpha signaling: the role of TNF-alpha in endochondral cartilage resorption. J Bone Miner Res. 2003;18(9):1584–92. doi:10.1359/jbmr.2003.18.9.1584.
83. Glass GE, Chan JK, Freidin A, Feldmann M, Horwood NJ, Nanchahal J. TNF-alpha promotes fracture repair by augmenting the recruitment and differentiation of muscle-derived stromal cells. Proc Natl Acad Sci U S A. 2011;108(4):1585–90. doi:10.1073/pnas.1018501108.
84. Cheng H, Jiang W, Phillips FM, Haydon RC, Peng Y, Zhou L, et al. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). J Bone Joint Surg Am. 2003;85-A(8):1544–52.
85. Wang X-X, Wang X, Li Z-L. Effects of mandibular distraction osteogenesis on the inferior alveolar nerve: an experimental study in monkeys. Plast Reconstr Surg. 2002;109(7):2373–83. http://www.ncbi.nlm.nih.gov/pubmed/12045565
86. Bodine PVN, Seestaller-Wehr L, Kharode YP, Bex FJ, Komm BS. Bone anabolic effects of parathyroid hormone are blunted by deletion of the Wnt antagonist secreted frizzled-related protein-1. J Cell Physiol. 2007;210(2):352–7. doi:10.1002/jcp.20834.
87. Guo J, Liu M, Yang D, Bouxsein ML, Saito H, Galvin RJS, et al. Suppression of Wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation. Cell Metab. 2010;11(2):161–71. doi:10.1021/ja8019214.Optimization.
88. Jilka RL, Brien CAO, Ali AA, Roberson P, Weinstein RS, Manolagas SC. Formation by actions on post-mitotic. Bone. 2010;44(2):275–86. doi:10.1016/j.bone.2008.10.037.INTERMITTENT.
89. Craft PD, Mani MM, Pazel J, Masters FW. Experimental study of healing in fractures of membranous bone. Plast Reconstr Surg. 1974;55(3):321–5.
90. Paccione MF, Warren SM, Spector JA, Greenwald JA, Bouletreau PJ, Longaker MT. A mouse model of mandibular osteotomy healing. J Craniofac Surg. 2001;12(5):444–50. doi:10.1097/00001665-200109000-00008.
91. Hasegawa T, Miwa M, Sakai Y, Nikura T, Lee SY, Oe K, et al. Mandibular hematoma cells as a potential reservoir for osteoprogenitor cells in fractures. J Oral Maxillofac Surg. 2012;70(3):599–607. doi:10.1016/j.joms.2011.03.043.
92. Oe K, Miwa M, Sakai Y, Lee SY, Kuroda R, Kurosaka M. An in vitro study demonstrating that haematomas found at the site of human fractures contain progenitor cells with multilineage capacity. J Bone Joint Surg Br. 2007;89-B(1):133–8. doi:10.1302/0301-620X.89B1.18286.
93. Spector JA, Luchs JS, Mehrara BJ, Greenwald JA, Smith LP, Longaker MT. Expression of bone morphogenetic proteins during membranous bone healing. Plast Reconstr Surg. 2001;107(1):124–34.
94. Steinbrech DS, Mehrara BJ, Rowe NM, Dudziak ME, Luchs JS, Saadeh PB, et al. Gene expression of TGF-beta, TGF-beta receptor, and extracellular matrix proteins during membranous bone healing in rats. Plast Reconstr Surg. 2000;105(6):2028–38.

95. Aronson J, Good B, Stewart C, Harrison B, Harp J. Preliminary studies of mineralization during distraction osteogenesis. Clin Orthop Relat Res. 1990a;(250):43–9. http://www.ncbi.nlm.nih.gov/pubmed/2293943
96. Aronson J, Good B, Stewart C, Harrison B, Harp J. Preliminary studies of mineralization during distraction osteogenesis. Clin Orthop Relat Res. 1990b;250:43–9.
97. Jazrawi LM, Majeska RJ, Klein ML, Kagel E, Stromberg L, Einhorn TA. Bone and cartilage formation in an experimental model of distraction osteogenesis. J Orthop Trauma. 1998;12(2):111–6.
98. Vauhkonen M, Peltonen J, Karaharju E, Aalto K, Alitalo I. Collagen synthesis and mineralization in the early phase of distraction bone healing. Bone Miner. 1990;10(3):171–81. http://www.ncbi.nlm.nih.gov/pubmed/2224204
99. Sato M, Yasui N, Nakase T, Kawahata H, Sugimoto M, Hirota S, et al. Expression of bone matrix proteins mRNA during distraction osteogenesis. J Bone Miner Res. 1998;13(8):1221–31. doi:10.1359/jbmr.1998.13.8.1221.
100. Yasui N, Sato M, Ochi T, Kimura T, Kawahata H, Kitamura Y, Nomura S. Three modes of ossification during distraction osteogenesis in the rat. J Bone Joint Surg Br. 1997;79(5):824–30. doi:10.1302/0301-620X.79B5.7423.
101. Li G, Virdi AS, Ashhurst DE, Simpson AH, Triffitt JT. Tissues formed during distraction osteogenesis in the rabbit are determined by the distraction rate: localization of the cells that express the mRNAs and the distribution of types I and II collagens. Cell Biol Int. 2000;24(1):25–33. doi:10.1006/cbir.1999.0449.
102. Karaharju EO, Aalto K, Kahri A, Lindberg L-A, Kallio T, Karaharju-Suvanto T, et al. Distraction bone healing. Clin Orthop Relat Res. 1993;297:38–43.
103. Hamanishi C, Yoshii T, Totani Y, Tanaka S. Lengthened callus activated by axial shortening. Clin Orthop Relat Res. 1994;307(307):250–4.
104. Ilizarov GA. Transosseous osteosynthesis. In: Green SA, editor. vol. 1. Berlin: Springer; 1992. doi:10.1017/CBO9781107415324.004
105. Cho T-J, Kim JA, Chung CY, Yoo WJ, Gerstenfeld LC, Einhorn TA, Choi IH. Expression and role of interleukin-6 in distraction osteogenesis. Calcif Tissue Int. 2007;80(3):192–200. doi:10.1007/s00223-006-0240-y.
106. Farhadieh RD, Dickinson R, Yu Y, Gianoutsos MP, Walsh WR. The role of transforming growth factor-beta, insulin-like growth factor I, and basic fibroblast growth factor in distraction osteogenesis of the mandible. J Craniofac Surg. 1999;10(1):80–6.
107. Holbein O, Neidlinger-Wilke C, Suger G, Kinzl L, Claes L. Ilizarov callus distraction produces systemic bone cell mitogens. J Orthop Res. 1995;13(4):629–38. doi:10.1002/jor.1100130420.
108. Lammens J, Liu Z, Aerssens J, Dequeker J, Fabry G. Distraction bone healing versus osteotomy healing: a comparative biochemical analysis. J Bone Miner Res. 1998;13(2):279–86. doi:10.1359/jbmr.1998.13.2.279.
109. Sato M, Ochi T, Nakase T, Hirota S, Kitamura Y, Nomura S, Yasui N. Mechanical tension-stress induces expression of bone morphogenetic protein (BMP)-2 and BMP-4, but not BMP-6, BMP-7, and GDF-5 mRNA, during distraction osteogenesis. J Bone Miner Res. 1999;14(7):1084–95. doi:10.1359/jbmr.1999.14.7.1084.
110. Rauch F, Lauzier D, Croteau S, Travers R, Glorieux FH, Hamdy R. Temporal and spatial expression of bone morphogenetic protein-2, -4, and -7 during distraction osteogenesis in rabbits. Bone. 2000;26(6):611–7.
111. Ashinoff RL, Cetrulo CL, Galiano RD, Dobryansky M, Bhatt KA, Ceradini DJ, et al. Bone morphogenic protein-2 gene therapy for mandibular distraction osteogenesis. Ann Plast Surg. 2004;52(6):585–90; discussion 591. http://www.ncbi.nlm.nih.gov/pubmed/15166991
112. Nuntanaranont T, Suttapreyasri S, Vongvatcharanon S. Quantitative expression of bone-related cytokines induced by mechanical tension-stress during distraction osteogenesis in a rabbit mandible. J Investig Clin Dent. 2014;5(4):255–65. doi:10.1111/jicd.12034.

113. Khanal A, Yoshioka I, Tominaga K, Furuta N, Habu M, Fukuda J. The BMP signaling and its Smads in mandibular distraction osteogenesis. Oral Dis. 2008;14(4):347–55. http://www.ncbi.nlm.nih.gov/pubmed/18449963
114. Mehrara BJ, Longaker MT. New developments in craniofacial surgery research. Cleft Palate Craniofac J. 1999;36(5):377–87. doi:10.1597/1545-1569(1999)036<0377:NDICSR>2.3.CO;2.
115. Pacicca DM, Patel N, Lee C, Salisbury K, Lehmann W, Carvalho R, et al. Expression of angiogenic factors during distraction osteogenesis. Bone. 2003;33(6):889–98. http://www.ncbi.nlm.nih.gov/pubmed/14678848
116. Carvalho RS, Einhorn TA, Lehmann W, Edgar C, Al-Yamani A, Apazidis A, et al. The role of angiogenesis in a murine tibial model of distraction osteogenesis. Bone. 2004;34(5):849–61. doi:10.1016/j.bone.2003.12.027.
117. Choi IH, Chung CY, Cho TJ, Yoo WJ. Angiogenesis and mineralization during distraction osteogenesis. J Korean Med Sci. 2002;17(4):435–47. doi:10.3346/jkms.2002.17.4.435.
118. Pérez-Sayáns M, Somoza-Martín JM, Barros-Angueira F, Rey JMG, García-García A. RANK/RANKL/OPG role in distraction osteogenesis. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol. 2010;109(5):679–86. doi:10.1016/j.tripleo.2009.10.042.
119. Zhu W-Q, Wang X, Wang X-X, Wang Z-Y. Temporal and spatial expression of osteoprotegerin and receptor activator of nuclear factor -kappaB ligand during mandibular distraction in rats. J Craniomaxillofac Surg. 2007;35(2):103–11. doi:10.1016/j.jcms.2006.12.001.
120. Fang TD, Salim A, Xia W, Nacamuli RP, Guccione S, Song HM, et al. Angiogenesis is required for successful bone induction during distraction osteogenesis. J Bone Miner Res. 2005;20(7):1114–24. doi:10.1359/JBMR.050301.
121. Aronson J. Temporal and spatial increases in blood flow during distraction osteogenesis. Clin Orthop Relat Res. 1994a;(301):124–31. http://www.ncbi.nlm.nih.gov/pubmed/8156663
122. Bragdon B, Lybrand K, Gerstenfeld L. Overview of biological mechanisms and applications of three murine models of bone repair: closed fracture with intramedullary fixation, distraction osteogenesis, and marrow ablation by reaming. Curr Protoc Mouse Biol. 2015;5:21–34. doi:10.1002/9780470942390.mo140166.
123. Matsubara H, Hogan DE, Morgan EF, Mortolock DP, Einhorn TA, Gerstenfeld LC. Vascular tissues are a primary source of BMP2 expression during bone formation induced by distraction osteogenesis. Bone. 2012;51(1):168–80. doi:10.1016/j.drugalcdep.2008.02.002.A.
124. Morgan EF, Hussein AI, Al-Awadhi BA, Hogan DE, Matsubara H, Al-Aql ZS, et al. Vascular development during distraction osteogenesis proceeds by sequential intramuscular arteriogenesis followed by intraosteal angiogenesis. Bone. 2012;51(3):535–45. doi:10.1016/j.bone.2012.05.008.Vascular.
125. Jacobsen KA, Al-Aql ZS, Wan C, Fitch JL, Stapleton SN, Mason ZD, et al. Bone formation during distraction osteogenesis is dependent on both VEGFR1 and VEGFR2 signaling. J Bone Miner Res. 2008;23(5):596–609. doi:10.1359/jbmr.080103.
126. Donneys A, Farberg AS, Tchanque-Fossuo CN, Deshpande SS, Buchman SR. Deferoxamine enhances the vascular response of bone regeneration in mandibular distraction osteogenesis. Plast Reconstr Surg. 2012a;129(4):850–6. doi:10.1097/PRS.0b013e31824422f2.
127. Farberg AS, Sarhaddi D, Donneys A, Deshpande SS, Buchman SR. Deferoxamine enhances bone regeneration in mandibular distraction osteogenesis. Plast Reconstr Surg. 2014;133(3):666–71. doi:10.1097/01.prs.0000438050.36881.a9.
128. Colnot C. Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. J Bone Miner Res. 2009;24(2):274–82. doi:10.1359/jbmr.081003.
129. Shibazaki R, Maki K, Tachikawa T, Shibasaki Y, Hinton RJ, Carlson DS, Opperman LA. Changes in parathyroid hormone-related protein and 3-dimensional trabecular bone structure of the mandibular condyle following mandibular distraction osteogenesis in growing rats. J Oral Maxillofac Surg. 2005;63(4):505–12. doi:10.1016/j.joms.2004.12.005.

130. Kasaai B, Moffatt P, Al-Salmi L, Lauzier D, Lessard L, Hamdy RC. Spatial and temporal localization of WNT signaling proteins in a mouse model of distraction osteogenesis. J Histochem Cytochem. 2012;60(3):219–28. doi:10.1369/0022155411432010.
131. Nott RL, Stelnicki EJ, Mack JA, Ben Y, Mitchell R, Mooney MP. Changes in the protein expression of hedgehog and patched-1 in perisutural tissues induced by cranial distraction. Plast Reconstr Surg. 2002;110(2):523–32. http://www.ncbi.nlm.nih.gov/pubmed/12142671
132. Lauterburg MT, Exner GU, Jacob HAC. Forces involved in lower limb lengthening: an in vivo biomechanical study. J Orthop Res. 2006;24(9):1815–22. doi:10.1002/jor.20217.
133. Aarnes GT, Steen H, Ludvigsen P, Waanders NA, Huiskes R, Goldstein SA. In vivo assessment of regenerate axial stiffness in distraction osteogenesis. J Orthop Res. 2005;23(2):494–8. doi:10.1016/j.orthres.2004.08.024.
134. Carter DR, Beaupre GS, Giori NJ, Helms JA. Mechanobiology of skeletal regeneration. Clin Orthop Relat Res. 1998;355S:S41–55.
135. Loboa EG, Fang TD, Parker DW, Warren SM, Fong KD, Longaker MT, Carter DR. Mechanobiology of mandibular distraction osteogenesis: finite element analyses with a rat model. J Orthop Res. 2005;23(3):663–70. doi:10.1016/j.orthres.2004.09.010.
136. Loboa EG, Fang TD, Warren SM, Lindsey DP, Fong KD, Longaker MT, Carter DR. Mechanobiology of mandibular distraction osteogenesis: experimental analyses with a rat model. Bone. 2004;34:336–43. doi:10.1016/j.bone.2003.10.012.
137. Shu Z, Xin-sheng C, Bing W. Mechanotransduction in osteoblast and osteocyte regulation***☆○◆. J Clin Rehabil Tissue Eng Res. 2011;15(24):4530–6.
138. Natu SS, Ali I, Alam S, Giri KY, Agarwal A, Kulkarni VA. The biology of distraction osteogenesis for correction of mandibular and craniomaxillofacial defects: a review. Dent Res J. 2014;11(1):16–26. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3955310&tool=pmcentrez&rendertype=abstract
139. Goodman CA, Hornberger TA, Robling AG. Bone and skeletal muscle: key players in mechanotransduction and potential overlapping mechanisms. Bone. 2015;80:24–36. doi:10.1016/j.bone.2015.04.014.
140. Klein-Nulend J, Bakker AD, Bacabac RG, Vatsa A, Weinbaum S. Mechanosensation and transduction in osteocytes. Bone. 2013;54(2):182–90. doi:10.1016/j.bone.2012.10.013.
141. Lanyon LE. Osteocytes, strain detection, bone modeling and remodeling. Calcif Tissue Int. 1993;53 Suppl 1:S102–6; discussion S106–7. http://www.ncbi.nlm.nih.gov/pubmed/8275362
142. Bonivtch AR, Bonewald LF, Nicolella DP. Tissue strain amplification at the osteocyte lacuna: a microstructural finite element analysis. Mater Eng. 2007;40(10):2199–206.
143. Han Y, Cowin SC, Schaffler MB, Weinbaum S. Mechanotransduction and strain amplification in osteocyte cell processes. Proc Natl Acad Sci U S A. 2004;101(47):16689–94. doi:10.1073/pnas.0407429101.
144. Davidson EH, Sultan SM, Butala P, Knobel D, Warren SM. Lacunocanalicular fluid flow transduces mechanical tension stress during distraction osteogenesis. J Craniofac Surg. 2013;24(5):1558–64. doi:10.1097/SCS.0b013e31828f2060.
145. Malone AMD, Anderson CT, Tummala P, Kwon RY, Johnston TR, Stearns T, Jacobs CR. Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. Proc Natl Acad Sci U S A. 2007;104(33):13325–30. doi:10.1073/pnas.0700636104.
146. Li J, Burr DB, Turner CH. Suppression of prostaglandin synthesis with NS-398 has different effects on endocortical and periosteal bone formation induced by mechanical loading. Calcif Tissue Int. 2002;70(4):320–9. doi:10.1007/s00223-001-1025-y.
147. Sawakami K, Robling AG, Ai M, Pitner ND, Liu D, Warden SJ, et al. The Wnt co-receptor LRP5 is essential for skeletal mechanotransduction but not for the anabolic bone response to parathyroid hormone treatment. J Biol Chem. 2006;281(33):23698–711. doi:10.1074/jbc.M601000200.
148. Kesavan C, Wergedal JE, Lau K-HW, Mohan S. Conditional disruption of IGF-I gene in type 1α collagen-expressing cells shows an essential role of IGF-I in skeletal anabolic response to loading. Am J Physiol Endocrinol Metab. 2011;301(6):E1191–7. doi:10.1152/ajpendo.00440.2011.

149. Lau K-HW, Baylink DJ, Zhou X-D, Rodriguez D, Bonewald LF, Li Z, et al. Osteocyte-derived insulin-like growth factor I is essential for determining bone mechanosensitivity. Am J Physiol Endocrinol Metab. 2013;305(2):E271–81. doi:10.1152/ajpendo.00092.2013.
150. Watanuki M, Sakai A, Sakata T, Tsurukami H, Miwa M, Uchida Y, et al. Role of inducible nitric oxide synthase in skeletal adaptation to acute increases in mechanical loading. J Bone Miner Res. 2002;17(6):1015–25. doi:10.1359/jbmr.2002.17.6.1015.
151. Hong P, Boyd D, Beyea SD, Bezuhly M. Enhancement of bone consolidation in mandibular distraction osteogenesis: a contemporary review of experimental studies involving adjuvant therapies. J Plast Reconstr Aesthet Surg. 2013;66(7):883–95. doi:10.1016/j.bjps.2013.03.030.
152. Buchman SR, Ignelzi MA, Radu C, Wilensky J, Rosenthal AH, Tong L, et al. Unique rodent model of distraction osteogenesis of the mandible. Ann Plast Surg. 2002;49(5):511–9. doi:10.1097/01.SAP.0000015490.10557.33.
153. Felice PA, Ahsan S, Perosky JE, Deshpande SS, Nelson NS, Donneys A, et al. Prophylactic amifostine preserves the biomechanical properties of irradiated bone in the murine mandible. Plast Reconstr Surg. 2014;133:314e–21e. doi:10.1097/01.prs.0000438454.29980.f8.
154. Deshpande SS, Gallagher KK, Donneys A, Nelson NS, Guys NP, Felice PA, et al. Stem cells rejuvenate radiation-impaired vasculogenesis in murine distraction osteogenesis. Plast Reconstr Surg. 2015;135(3):799–806. doi:10.1097/PRS.0000000000001024.
155. Fregene A, Jing XL, Monson LA, Buchman SR. Alteration in volumetric bone mineralization density gradation patterns in mandibular distraction osteogenesis following radiation therapy. Plast Reconstr Surg. 2009;124(4):1237–44. doi:10.1097/PRS.0b013e3181b5a42f.
156. Schwarz DA, Jamali AM, Kakwan MS, Fregene A, Arman KG, Buchman SR. Biomechanical assessment of regenerate integrity in irradiated mandibular distraction osteogenesis. Plast Reconstr Surg. 2009;123(2 Suppl 1):114S–22S. doi:10.1097/PRS.0b013e318191c5d2.
157. Tchanque-Fossuo CN, Monson LA, Farberg AS, Donneys A, Zehtabzadeh AJ, Razdolsky ER, Buchman SR. Dose-response effect of human equivalent radiation in the murine mandible: part I. A histomorphometric assessment. Plast Reconstr Surg. 2011;128(1):114–21. doi:10.1097/PRS.0b013e31821741d4.
158. Deshpande SS, Gallagher KK, Donneys A, Tchanque-Fossuo CN, Sarhaddi D, Nelson NS, et al. Parathyroid hormone therapy mollifies radiation-induced biomechanical degradation in murine distraction osteogenesis. Plast Reconstr Surg. 2013a;132(1):91–100. doi:10.1097/PRS.0b013e3182910ae7.
159. Deshpande S, James AW, Blough J, Donneys A, Wang SC, Cederna PS, et al. Reconciling the effects of inflammatory cytokines on mesenchymal cell osteogenic differentiation. J Surg Res. 2013b;185(1):278–85. doi:10.1016/j.jss.2013.06.063.
160. Tchanque-Fossuo CN, Donneys A, Razdolsky ER, Monson LA, Farberg AS, Deshpande SS, et al. Quantitative histologic evidence of amifostine-induced cytoprotection in an irradiated murine model of mandibular distraction osteogenesis. Plast Reconstr Surg. 2012;130(6):1199–207. doi:10.1097/PRS.0b013e31826d2201.