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THE LIFE OF A FRACTURE: BIOLOGIC PROGRESSION, HEALING GONE AWRY, AND EVALUATION OF UNION

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Abstract

» New knowledge about the molecular biology of fracture-healing provides opportunities for intervention and reduction of risk for specific phases that are affected by disease and medications.

» Modifiable and nonmodifiable risk factors can prolong healing, and the informed clinician should optimize each patient to provide the best chance for union.

» Techniques to monitor progression of fracture-healing have not changed substantially over time; new objective modalities are needed.

Background and Epidemiology

Skeletal injuries comprise an important socioeconomic burden in the United States, with 12 to 15 million fractures annually, resulting in lost wages and functional impairment until healing occurs[1]. While many fractures will heal successfully, up to 10% proceed to nonunion, a clinical state that causes additional morbidity, prolonged recovery, and expenses to the medical system[2,3]. Establishing a diagnosis of nonunion is difficult because the essence of nonunion is an inappropriate biologic response to pathology over the time course of normal fracture-healing. Prolonging interventions for nonunion can result in additional pain, disability, and uncertainty that imparts a burden on a patient's quality of life.

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Fracture care employs fundamental principles of stabilization to support mobilization and encourage immediate weight-bearing with many lower-extremity fractures. Fractures heal through 2 primary pathways, with the contribution of each depending on the strain profile and the biologic microenvironment at the fracture site. Primary bone-healing, or intramembranous ossification, occurs along the periosteal surface adjacent to the fracture and across minimal fracture gaps when absolute stability of <2% of mechanical strain is achieved[4]. The process of intramembranous ossification was originally described by Robert Danis as "self-welding"[5] to describe haversian remodeling and migration of lamellar bone across these compressed stable fractures with a minimal gap[6]. Secondary bone-healing, or endochondral ossification, is the predominant form of healing in the majority of diaphyseal fractures. While intramembranous repair still occurs to seal off the ends of the bone and along the periosteal surfaces of the bone adjacent to the gap, a cartilaginous callus forms within the fracture gap, and the process of endochondral ossification describes mineralization and remodeling of the cartilage into bone. The cartilaginous callus thus aims to provide intermediate stability across the fracture gap during bone healing; John Charnley stated that "nature has thus done its own internal fixation."[7] Multiple microenvironmental factors drive osteochondral progenitors to a chondrogenic fate, including increased micromotion at the fracture site associated with larger gap size and mechanical strains between 2% and 10%, decreased oxygen tension, and lack of vascularization[8,9]. In order for bone to successfully heal, a series of properly timed molecular interactions must occur at the fracture site and in concert with systemic biology. Understanding biologic contributions and how they are affected by various risk factors can help guide individual treatment plans for patients and better predict the risk of nonunion. This review will focus on diaphyseal endochondral ossification and describe the phases of healing along with the perturbations and the opportunities for augmentation of each phase.

Phases of Bone-Healing

Inflammatory Phase

The inflammatory phase is classically considered the first phase of fracture-healing. At the time of fracture, intracortical, endosteal, and periosteal vessels are sheared, producing a fibrin-rich hematoma. Evacuation of the hematoma at 4 to 7 days after fracture or repeated irrigation and debridement of the fracture site can delay bone-healing, but is often necessary to debride devitalized tissue in order to reduce the risk of infection[10,11]. Chemokines that are released from activated platelets within the hematoma promote the migration of macrophages and neutrophils to the fracture[12] (Fig. 1). These cells debride devitalized tissue and promote the recruitment of inflammatory cells through the release of proinflammatory cytokines that include tumor necrosis factor-alpha (TNF-α) and interleukins-1 and 6 (IL-1 and IL-6)[13–16] (Fig. 2). This proinflammatory phase is important for the recruitment of progenitor cells that originate from the periosteum, the bone marrow, the soft tissue, and the systemic circulation. Proliferation and differentiation of these progenitor cells is modulated by the release of growth factors that include bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF) from the inflammatory cells[13–16].

Careful cellular resolution of the inflammatory phase, through a shift from a proinflammatory macrophage (M1)-dominant state to the pro-reparative anti-inflammatory macrophage phenotype (M2), is critical to ensure proper healing[17–19]. This process is regulated through a complex positive feedback loop such that depletion of the macrophages or disruption of the initial proinflammatory signaling pathways will delay proper healing[9]. However, subsequent modulation of the inflammatory state to promote an M2 phenotype may be a potential target for augmentation of bone-healing. Stem cells are known to have a powerful anti-inflammatory secretome that likely plays a role in the resolution of inflammation. The biochemical milieu also influences macrophage polarity, and recent work suggests that endogenous or exogenous BMPs at the fracture site can promote M2 differentiation[20–22].

Systemic inflammatory conditions, such as diabetes mellitus and rheumatoid arthritis, exhibit prolonged healing responses and elevation of inflammatory cytokines at the fracture site, suggesting that excessive inflammation, at least partially, contributes to delayed repair[23–25]. Failure to resolve inflammation in these states may impair later healing processes, including angiogenesis, osteoclast recruitment, and deposition of bone; however, the exact mechanisms of chronic inflammation that cause delayed bone-healing are not completely understood[26].

Debate exists regarding the effects of anti-inflammatory medication on fracture-healing. Prostaglandins released from inflammatory cells have a multitude of positive effects on subsequent phases of fracture-healing to promote bone formation[27]. Animal studies have demonstrated that cyclooxygenase-2 (COX-2) inhibition with nonsteroidal anti-inflammatory drugs (NSAIDs) or gene knockout will impair healing. Decreased expression of COX-2 has been observed in the fractures of older mice, which may partially explain prolonged healing with aging[28]. Limited human data suggest that there may be a dose-dependent relationship with prolonged NSAID use, but data currently remain inconclusive[29–32]. Similarly, long-term corticosteroid use can delay bone-healing, but through more complex mechanisms than just the anti-inflammatory effects[33–35]. There exists a growing body of literature that NSAID use at clinically relevant dosing likely does not impair fracture-healing, and this supports the use of NSAIDs in the perioperative period without conferring additional nonunion risk[36–40]. NSAIDs should be used judiciously in patients with suspected risk factors for nonunion to avoid a potential compounding effect[41].

Fibrovascular Phase

The next phase of healing is directed toward angiogenesis, stem cell recruitment, proliferation, and differentiation. The process of angiogenesis is critical during fracture repair as it provides a source of cells, oxygen, nutrients, and waste removal for the healing tissue. Angiogenesis relies on multiple signaling molecules and their receptors, most notably vascular endothelial growth factor (VEGF)[9,42]. Many animal studies have implicated hypoxia and poor blood flow as direct causes of delayed union, and blockade of the VEGF receptor with bevacizumab has directly delayed healing[43–45]. Diseases associated with delayed healing, including diabetes mellitus, demonstrate decreased levels of VEGF and angiogenesis, which can be reversed by treatment with TNF-α inhibitors[46]. Interestingly,

atrophic nonunion tissue has a similar content of VEGF expression and vascularity to normal healing tissue; thus, it is likely that an unbalanced ratio between vasculogenic and osteogenic factors contributes to impaired healing[47,48].

The osteochondral progenitor cells that give rise to the fracture callus are recruited locally from the bone (the endosteum and the periosteum), the surrounding soft tissue, the bone marrow, and the circulation[49–52]. Periosteal and endosteal stem cells within the bone both make a contribution to the fracture callus[53,54]. As such, the functional consequence of surgical techniques that disrupt either of these populations should be considered. Aggressive soft-tissue handling, including periosteal stripping and muscular manipulation, impairs fracture-healing by altering blood supply, but it is likely that evacuation of stem cells is at least partially responsible as well[51,55]. Although intramedullary reaming has been shown to disrupt valuable endosteal blood supply, the Study to Prospectively Evaluate Reamed Intramedullary Nails with Tibial Fractures (SPRINT) trial prospectively demonstrated that reaming for tibial nails may instead decrease the rate of nonunion[56,57]. Interestingly, intramedullary reaming also increases marrow progenitor cells at the fracture site and reduces inflammatory cytokines, effectively pushing the progression of healing to the fibrovascular phase on postoperative day zero[58]. In the setting of hypertrophic nonunion, undifferentiated stem cells contained within the fracture callus maintain their ability to differentiate to osteogenic or chondrogenic cells, which may offer a target for nonunion intervention[59].

Bone Formation

In addition to the direct differentiation of progenitor cells to bone during intramembranous bone repair, chondrocytes within the fracture gap give rise directly to bone through endochondral repair[60] (Fig. 3). Conversion of cartilage to bone begins with proliferation and hypertrophic maturation of the chondrocytes in order to create a temporary bridge across the bone gap[61]. These hypertrophic chondrocytes are highly bioactive and secrete angiogenic factors, including VEGF, PDGF, and placental growth factor (PlGF), along with nerve growth factor (NGF), to recruit the neurovascular bundle into the avascular, aneural cartilage anlagen[62,63]. Vascular invasion causes mineralization of the cartilage matrix through secretion of the osteogenic promoters BMP and Wnt[60,64–67]. At this point, the unique cellular and mechanical microenvironment seems to support phenotypic plasticity within the chondrocytes so that they can either become osteoblasts that form the new hard callus, undergo apoptosis to form the marrow cavity, or potentially dedifferentiate to give rise to osteochondral progenitors in the bone-lining tissue through a conserved process that recently has been defined as palingenesis[60,68,69].

Clinical opportunities to augment fracture-healing have focused on 3 critical molecular pathways that are known to regulate chondrogenesis and osteogenesis at various phases of repair: BMPs, Wnt/β-catenin, and parathyroid hormone (PTH) derivatives. BMPs are canonical osteogenic proteins that are required for effective fracture repair and are expressed by inflammatory cells, vascular endothelial cells, and muscle stem cells to modulate to fracture repair[65,66,70–72]. Interestingly, BMPs play an important role in both intramembranous and endochondral fracture-healing. This potent osteogenic effect has led to

the development of BMP-2 as a therapeutic agent. While predominantly approved for use in spinal fusion, BMP-2 does have U.S. Food and Drug Administration (FDA) approval for the narrow indication window of surgical implantation involving acute open tibial shaft fractures that are stabilized with an intramedullary nail and treated within 14 days of the initial injury. Off-label application has been used to treat other types of fractures, and clinical evidence for efficacy was nicely summarized by Nauth et al.[73] (Fig. 4). This success is balanced by the high product costs and reports of severe side effects, including heterotopic ossification and cancer[74].

Similarly, Wnt/β-catenin signaling has a well-established osteogenic role and is important during both the intramembranous and endochondral phases of fracture-healing[75]. Dysregulation of this pathway can contribute to impaired fracture-healing and has been associated with age-related delayed healing[76]. Binge alcohol exposure prior to fracture has been shown to decrease β-catenin levels in mice and may contribute to poor healing outcomes that are associated with alcohol abuse[77]. Conversely, therapeutic activation is being explored as a novel pathway to promote healing. Interestingly, activation of Wnt/β-catenin by low-dose lithium administration improves callus mineralization and torsional strength and can attenuate the effects of alcohol exposure[78,79]. More recently, there has been clinical interest in the use of the romosozumab antibody that blocks the Wnt/β-catenin inhibitor sclerostin in order to indirectly increase signaling. While early evidence showed enhanced bone mass and strength after fracture in animal studies, follow-up studies have not demonstrated the same efficacy in large human trials[80–85].

Teriparatide and abaloparatide are promising PTH and PTH-related protein (PTHrP) analog drugs that may be able to enhance fracture-healing[86]. PTH and PTHrP function to maintain calcium homeostasis via a negative feedback loop and promote normal cartilage maturation during endochondral ossification. Animal studies clearly show that intermittent PTH therapy promotes fracture-healing and suggest a strong therapeutic potential of PTH[87]. Mechanistically, PTH therapies result in proliferation of the fracture callus progenitors and enhanced soft callus formation that ultimately lead to accelerated union rates and increased mechanical strength due to improved bone mineral quality[88–93]. Clinical studies offer a less certain view of efficacy, partly due to variability in doses, fracture sites, and patient age, with much more limited data comparing the efficacy of teriparatide and abaloparatide[86,87,94]. Future studies will be necessary to further delineate the therapeutic potential of PTH/PTHrP analogs for fracture-healing in humans.

Widespread prevalence of hypovitaminosis D raises concern for delays in fracture-healing[95]. Vitamin D has been implicated in every stage of fracture-healing, but most importantly during the mineralization phase[96]. Although deficiency is associated with nonunion, decreased bone turnover, and increased fracture risk, there is mixed evidence as to the clinical importance of vitamin D as a causal factor for nonunion[97,98]. Supplementation in fracture cases can increase bone mineral density and callus area; however, no universal guidelines for vitamin D supplementation exist[99]. A recent survey among members of the Orthopaedic Trauma Association (OTA) and the Canadian Orthopaedic Trauma Association (COTA) established that the most common dosing strategies in fracture patients were 1,000 IU daily (14.6%) and 2,000 IU daily (13.4%) among fellowship-trained traumatologists,

demonstrating little to no agreement[99]. A systematic review concluded that while vitamin D supplementation clearly increases 25-hydroxyvitamin D (25[OH]D) serum levels, no studies exist that definitively demonstrate that this impacts fracture-healing[100].

Remodeling

Hard callus remodeling is the final phase of fracturere repair and can continue for several years. Haversian remodeling is orchestrated in part by osteoclasts to exchange woven to lamellar bone. Osteoporosis medications, such as bisphosphonates or the RANKL (receptor activator of nuclear factor-κB ligand) inhibitors denosumab and osteoprotegerin, delay remodeling in animal studies and cause changes in the material properties of fracture callus, including increased strength and decreased ultimate stress[101,102]. Meta-analyses of human studies have not demonstrated a delay in healing with bisphosphonate use; thus, many recommend the continuation of osteoporosis medication after a fracture because the risk of secondary fracture outweighs the potential consequence for fracture-healing[103–105]. There is a known phenomenon with prolonged bisphosphonate use that results in deranged remodeling, leading to osteonecrosis of the jaw and stress fractures, most notably in the subtrochanteric femoral region. These occur in <5% of bisphosphonate users and have proven difficult to heal[106]. The American Society for Bone and Mineral Research recommends reevaluation after 3 to 5 years of use of bisphosphonates to minimize these complications[107].

Defining Nonunion

Disruption or failure of the normal cascade of fracture-healing will result in bone nonunion. The FDA defines a nonunion as any fracture that is 9 months old and has not shown evidence of fracture-healing in 3 months[108]. Clinicians utilize subjective patient reports, serial physical examinations, and radiographic evidence of mineralization across the fracture site to help determine union; however, the fracture-healing timeline and definitions are not universally agreed upon in the orthopaedic community[109,110]. This variability also exists in the European community, with even less consensus between orthopaedic and trauma surgeons who both treat fracture nonunions[111].

Nonunions can be classified as hypertrophic, atrophic, or oligotrophic. Hypertrophic nonunions form a soft callus that fails to convert to bone due to excessive micromotion at the fracture site from lack of proper stabilization. The fracture callus is typically enlarged on radiographs and is unstable to weight-bearing. Atrophic nonunions present because of inadequate formation of bone in the callus, and often are attributable to inadequate micromotion at the fracture site and/or a suboptimal local or systemic healing environment[112]. Atrophic nonunions were originally thought to be formed by poor vascular flow to the fracture site. Recent studies have shown that atrophic non-union tissue contains similar vascular density to hypertrophic nonunions and normally healing bone, although the role of vascular contribution is not completely understood[113]. Oligotrophic nonunions fit neither criterion and are not hypertrophic in their tissue response, nor are they avascular; they often occur with inappropriate alignment and proximity of the fracture fragments. Both atrophic and hypertrophic tissue retain the ability to differentiate into

osteogenic, chondrogenic, and adipogenic cells in vitro; however, atrophic tissue has much less osteogenic potential and greater senescence, and it can impair the differentiation of surrounding cells[59,114].

Risk Factors for Developing Nonunion

Nonmodifiable Risk Factors

Injury severity is the strongest predictor of nonunion. In a nationwide study of 309,330 patients, the number of fractures healing at 1 time point was most predictive for nonunion, with an odds ratio of 2.65[40]. A systematic review by Santolini et al. found that an open fracture and a need for open reduction were the variables with the highest level of evidence[115]. Aside from osseous involvement, soft-tissue injury produces a compromised local environment that impairs bone-healing[116,117]. Soft-tissue injury that is severe enough to warrant flap coverage or fasciotomy for compartment syndrome can increase the risk of nonunion up to 20%[118,119]. Additional wound complications, including infections, can compromise fracture-healing[120], although an in-depth discussion of septic nonunion is outside the scope of this article. Concomitant thoracic and hemorrhagic injuries are common in polytrauma fracture cases. Well-controlled animal studies have demonstrated an enhanced inflammatory response and impaired bone and callus formation after fracture with hemorrhagic shock[121–123]. Subsequent resuscitation produces a larger callus size and a prolonged remodeling phase, suggesting that resuscitation may confer additional benefits for trauma patients in these cases[124]. Interestingly, traumatic brain injury is observed to enhance fracture-healing in patients with polytrauma, although the neuro-inflammatory mechanisms of this phenomenon are not completely understood[125]. Recent controlled animal studies demonstrated that healing responses and serum inflammatory profiles were more robust when fractures occurred contralateral to brain injury, suggesting the importance of neuronal crossover in modulating healing[126].

The impact of age on fracture-healing is not well understood. Elderly patients have an elevated risk of sustaining a fracture, and healing may progress more slowly than in younger patients, perhaps because of osteoporosis[127]. Age has been demonstrated as an independent risk factor for clavicle nonunion in patients between the ages of 20 and 46 years[128]. However, examination of >47,000 Medicare patients revealed that more elderly (>75-year-old) patients have a surprisingly lower rate of nonunion compared with their younger (<69-year-old) counterparts, suggesting that other factors such as mobility or injury severity may have primacy[129]. Although there are no remarkable differences in the relative expression of genes or the ratio of cartilage to bone in fracture callus of elderly mice, overall callus size is decreased[130]. These findings are likely due to inflammatory response derangement, decreased vascularity, and poor proliferation and differentiation of cells[131–134]. Although the molecular and cellular mechanisms for delayed bone-healing in older patients are not fully understood, additional research has begun to address this complex issue[135–138].

Modifiable Risk Factors

Systemic factors impact bone-healing, and patients must optimize modifiable behaviors to decrease the risk of non-union (Fig. 5). Obesity alone has not been shown to be an independent risk factor for fracture-healing in human studies, but animal studies have demonstrated that obesity increases risk of delay in union and is associated with an exaggerated inflammatory response after fracture[139–142]. Diabetes is a common comorbidity of obesity and prolongs bone-healing through impaired vascularization, immune dysregulation, and poor callus mineralization[143]. Patients with diabetes have demonstrated higher rates of nonunion after ankle fractures, whether treated operatively or non-operatively, and this phenomenon remains uninvestigated in other injuries[144,145]. In animal studies, administration of insulin locally can help rescue this phenotype and return fractures to a normal healing trajectory[139,140,146–148]. Interestingly, obese patients can be malnourished, resulting in metabolic derangements that delay fracture-healing[149]. The correction of metabolic or endocrine abnormalities should be addressed in every patient as it can be sufficient to promote union in patients with nonunion[150,151].

Smoking status is the lifestyle factor with the greatest amount of evidence that is correlated with risk of nonunion[115]. In a systematic review, Pearson et al. found that smokers have a 2.2-fold higher risk of developing a nonunion and display prolonged healing times after nonunion surgery[152]. Others have correlated this latter finding with greater amounts of pain as well as disability[153]. Among a variety of other perturbations in the healing cascade, inhibition of stem cell migration by nicotine is at least partially responsible for this delay[154]. Smoking cessation is arguably the most important intervention a patient can undergo to decrease the risk of nonunion after fracture. In smokers, cessation for the first 6 weeks after fracture surgery has been shown to significantly decrease the risk of postoperative complications[155].

Many medications interact with fracture biology; however, few are more widely utilized in the fracture setting for pain management than opioids and NSAIDs. The effects of NSAIDs have long been investigated, but few studies have investigated the risk of nonunion with opioid use. The epidemic of opioid abuse has brought to light the dangers of overprescribing; however, opioids are still an effective pain medication[156]. In a retrospective review of 309,330 fractures, opioid use was associated with prolonged healing, even when controlling for age, sex, number of fractures, and smoking status, and the risk of nonunion was almost double for chronic opioid users[157]. Animal studies demonstrated that those that were treated with opioids had smaller callus volume and delayed maturation of callus after 8 weeks compared with healthy controls[158]. There are little human data on the causal relationship between opioid use and nonunion; perhaps increased cultural acceptance of multimodal analgesia will allow for comparative studies.

Military Extremity Trauma

Ongoing military conflicts have led to an increased incidence of combat-related traumatic injuries[159,160]. Over 75% of modern war injuries involve the extremities, with contaminated open fractures and bone and tissue loss from explosive devices[160,161]. Complications in fracture-healing, such as delayed union or nonunion, are estimated to occur in approximately

10% to 20% of normal civilian injuries. In contrast, nonunion rates as high as 50% have been reported at 1 year after injury for open tibial fractures that were sustained during combat, in part due to an increased occurrence of infection[162,163]. Unfortunately, a return-to-duty rate of only 22% is reported for American soldiers with isolated type-III open tibial fractures, which is less than half of the return-to-work rate for similarly severe injuries in the civilian population[164,165]. These injuries also lead to emotional and psychiatric dysfunction, and less than half of soldiers sustaining high-energy extremity trauma ever resume civilian employment[161].

Monitoring Fracture-Healing

The measurement of fracture-healing remains an unsolved clinical problem; there is no universal consensus on the best method to make this assessment in a quantitative fashion[166–176].

Clinical Assessment

Clinical assessment is commonly relied upon as a gross measurement of fracture-healing, despite poor reliability and subjectivity[177]. The most common assessment methods are presence of pain, tenderness with palpation, and ability to bear weight on the affected limb[178]. Patient-reported scoring systems reflect functional capabilities and restrictions but are unable to directly assess fracture biology. In low-resource settings, such as in low and middle-income countries, clinical judgment is sometimes the only available method[179]. New and simple clinical instruments, such as the squat-and-smile test, may provide outcome predictions with strong interrater reliability[180].

Imaging

Serial radiography is the most common method to track fracture-healing over time, but this method is subjective and fallible. Experts blinded to other clinical information struggle to gauge healing status and can change their opinion in 40% of cases when unblinded[181–183]. Opinion varies between radiologists and orthopaedic surgeons analyzing radiographs; the use of standardized scoring systems can help decrease this discrepancy[109,181]. The Radiographic Union Score for Tibial Fractures (RUST) was developed in 2010 to standardize qualitative radiographic scoring[166]. The RUST score grades callus progression on 4 cortices and correlates with callus strength and rigidity. In a large validation study, 90% of orthopaedic traumatologists agreed that a score of 10 on the RUST and 13 on the modified RUST should correlate with union[167]. The ability to objectively and qualitatively measure fracture-healing is an improvement over subjective opinion, increasing intraobserver reliability. Similar methodology can be used in metadiaphyseal tibial, hip, and distal radial fractures[168,184]. Evidence of cortical bridging and use of these scoring systems demonstrate strong interrater reliability[169,185,186]. The Nonunion Risk Determination tool is a predictive model that is based on 7 factors that are associated with tibial nonunion, primarily depending on a strong association of patient health and the severity of injury with nonunion[170].

Radiographic scoring systems remain indirect assessments of fracture biology and are limited in their efficacy. Assessment is difficult during the initial phases of fracture-healing before mineralization, as well as when implants obscure the view of the fracture site[171]. New computerized algorithms can predict stages of healing and mechanical strength based on radiographs, which may help improve reliability[187]. Other imaging modalities can be helpful to determine if a fracture has failed to heal, including computed tomography (CT), ultrasound, and nuclear medicine; however, these modalities carry their own risks and are not commonly used to monitor early fracture-healing[172,188]. Implantable smart devices, such as plates and intramedullary nails, are being developed to provide telemetric information of strain through a fracture[189,190]. These devices allow for live assessment of biomechanical properties during the formation of soft callus and conversion to bone and are correlated with histologic and micro-CT data of bone-healing in preclinical models[191].

Serologic Markers

A serum biomarker could allow for the quantification of fracture-healing. Identification of a marker with stable basal levels in circulation and perturbation specific to only fracture-healing has yet to be definitively established. Many have been investigated but are nonspecific and are involved in immunologic cascades, growth factors, markers of bone turnover, and cellular signaling molecules[173–175]. Many vary by age, sex, and metabolic or endocrine derangement, which is found in up to 84% of patients with nonunion, limiting their use as a specific biomarker[150]. Unsuccessful attempts have been made to correlate TGF-β with fracture-healing[192]. Proteins associated with osteoclast activity such as tartrate-resistant acid phosphatase 5b and C-terminal cross-linking telopeptide of type-I collagen have shown greater promise to evaluate nonunion, but they are not useful in the early phases to predict a healing trajectory[193]. The use of many biomarkers consecutively in a predictive algorithm is potentially more optimal. A recent proteomic study demonstrated time-dependent changes in 850 proteins, which could be clustered throughout phases of fracture-healing to yield 50 candidate biomarkers[194]. At this time, it is not feasible to characterize the proteome of every patient with a fracture at every visit, but these data provide a map to further investigate combinations that can guide a predictive model of fracture-healing.

One promising candidate that is specific for cartilage to bone conversion has emerged: collagen X. This extracellular matrix protein is synthesized by hypertrophic chondrocytes during endochondral fracture-healing and is associated with vascularization and mineralization of the cartilaginous callus[195]. Recently, a repeatable and reliable assay has been validated for the measurement of a collagen X degradation fragment from serum (“CXM”)[196]. This biomarker was tested primarily in the skeletally immature in order to predict growth trajectories, but a small series with 3 patients showed strong correlation between CXM and progression of fracture-healing[196]. Preclinical data demonstrated that this collagen X biomarker correlates with normal fracture-healing in male and female mice and that the kinetics of the biomarker appropriately correlated with gene expression and histomorphometric quantification of fracture callus composition[176]. Clinically, this promising avenue remains in development.

Overview

Current definitions and assessment of union are inconsistent and vary based on expert opinion. There exists a clinical need for a superior assessment method to quantify fracture-healing and define union. The biology of fracture-healing compels us to optimize modifiable patient factors to provide the best opportunity for union, which is best achieved through local and systemic biology working in conjunction with biomechanical stability. Circulating biomarkers are appealing future targets for clinical development to track progress, but to date, none have been validated or are specific for fractures. Additional research is required to identify an accurate and reliable measurement of union.

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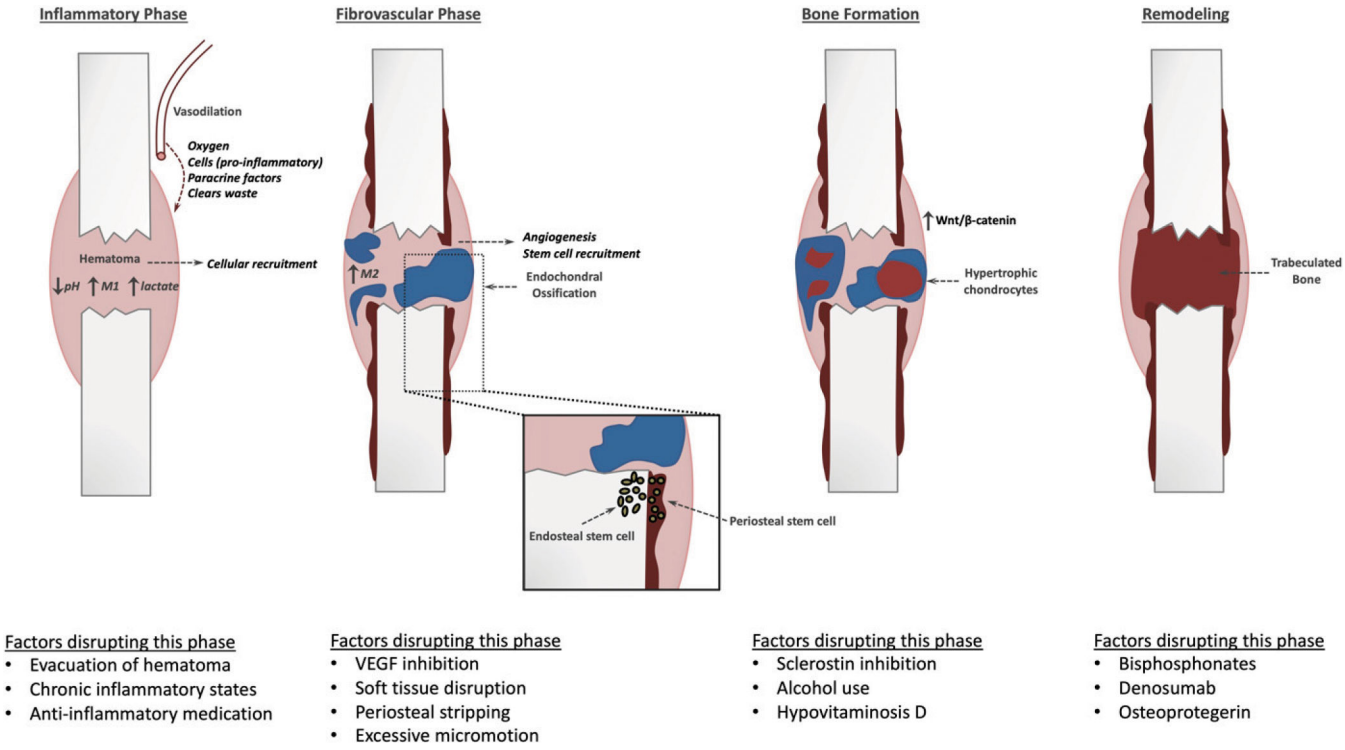

Fig. 1.

The key steps of endochondral ossification during fracture-healing and examples of common perturbations that disrupt the respective phases. VEGF 5 vascular endothelial growth factor. (Reproduced, with modification, from: Bahney CS, Hu DP, Miclau T III, Marcucio RS. The multifaceted role of the vasculature in endochondral fracture repair. Front Endocrinol [Lausanne]. 2015;6:4, under Open Access License CC BY 4.0.)

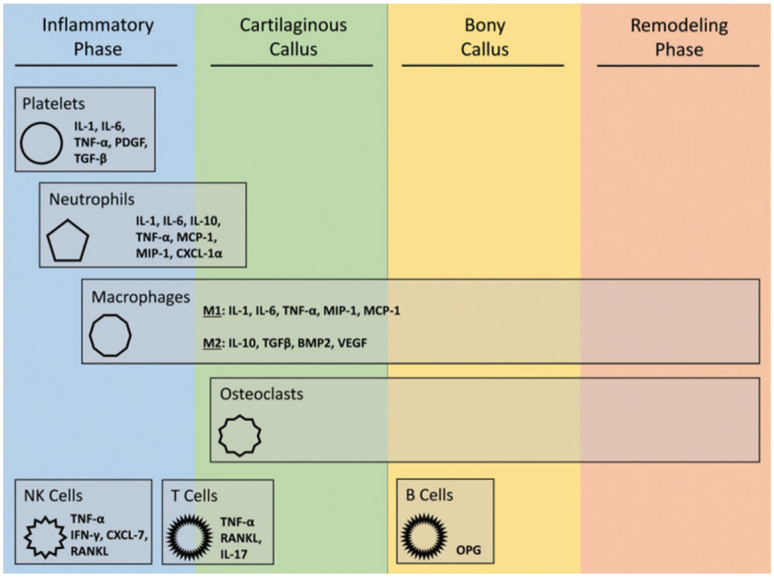

Fig. 2.

The role of immune cells during fracture repair[14]. Bone fracture-healing can be viewed as a 4-stage process. Immune cells play important roles throughout this process; however, a majority of their activity occurs during the early stages of fracture-healing. IL = interleukin, TNF-α = tumor necrosis factor-alpha, PDGF = platelet-derived growth factor, TGF-β = transforming growth factor-beta, MCP1 = monocyte chemoattractant protein-1, MIP-1 = macrophage inflammatory protein-1, CXCL = C-terminal crosslinking telopeptide of type-I collagen, BMP2 = bone morphogenetic protein-2, VEGF = vascular endothelial growth factor, NK = natural killer, IFN-γ = interferon gamma, RANKL = receptor activator of nuclear factor-κB ligand, and OPG = osteo-protegerin. (Reproduced from: Baht GS, Vi L, Alman BA. The role of the immune cells in fracture healing. Curr Osteoporos Rep. 2018 Apr;16[2]:138–45, under Creative Commons Attribution 4.0 International License.)

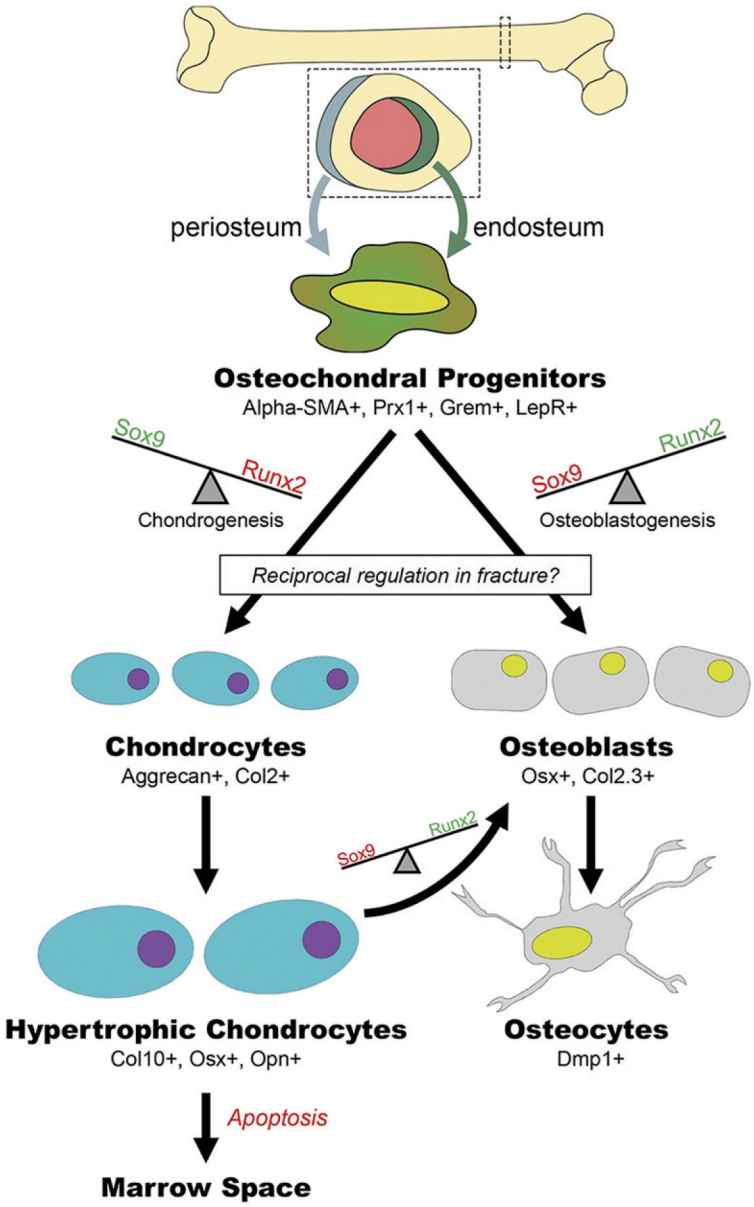

Fig. 3.

Chondrocyte to osteoblast transformation. Alpha-SMA = alpha smooth muscle actin. (Reproduced from: Bahney CS, Zondervan RL, Allison P, Theologis A, Ashley JW, Ahn J, Miclau T, Marcucio RS, Hankenson KD. Cellular biology of fracture healing. J Orthop Res. 2019 Jan;37[1]:35–50.)

Recommendation	Grade of Recommendation
The acute or delayed use of BMPs in open fractures is safe.	A
There is inconclusive/insufficient evidence to support the use of BMPs acutely in the management of all types of open tibia fractures.	I
There is good evidence to support the acute use of BMPs in severe (types IIIA and IIIB) open tibia fractures. The evidence supports that such use will reduce rates of reintervention, lower infection rates, and is cost-effective.	B
There is level 1 evidence from a single study that supports the use of BMPs combined with allograft as an alternative to autogenous bone grafting in the delayed reconstruction of bone defects secondary to open fracture.	B

[a] A = good evidence from Level I studies with consistent findings;
B = fair evidence from Level II or III studies with consistent findings,
C = Poor-quality evidence from Level IV or V studies with consistent findings,
I = insufficient or conflicting evidence.[30]

Fig. 4.

Summary of grades of recommendation for BMP use in open fractures[73]. (Reprinted from: Injury 40[Suppl 3], Nauth A, Ristiniemi J, McKee MD, Schemitsch EH. Bone morphogenetic proteins in open fractures: past, present, and future, p S27–31, Copyright 2009, with permission from Elsevier.)

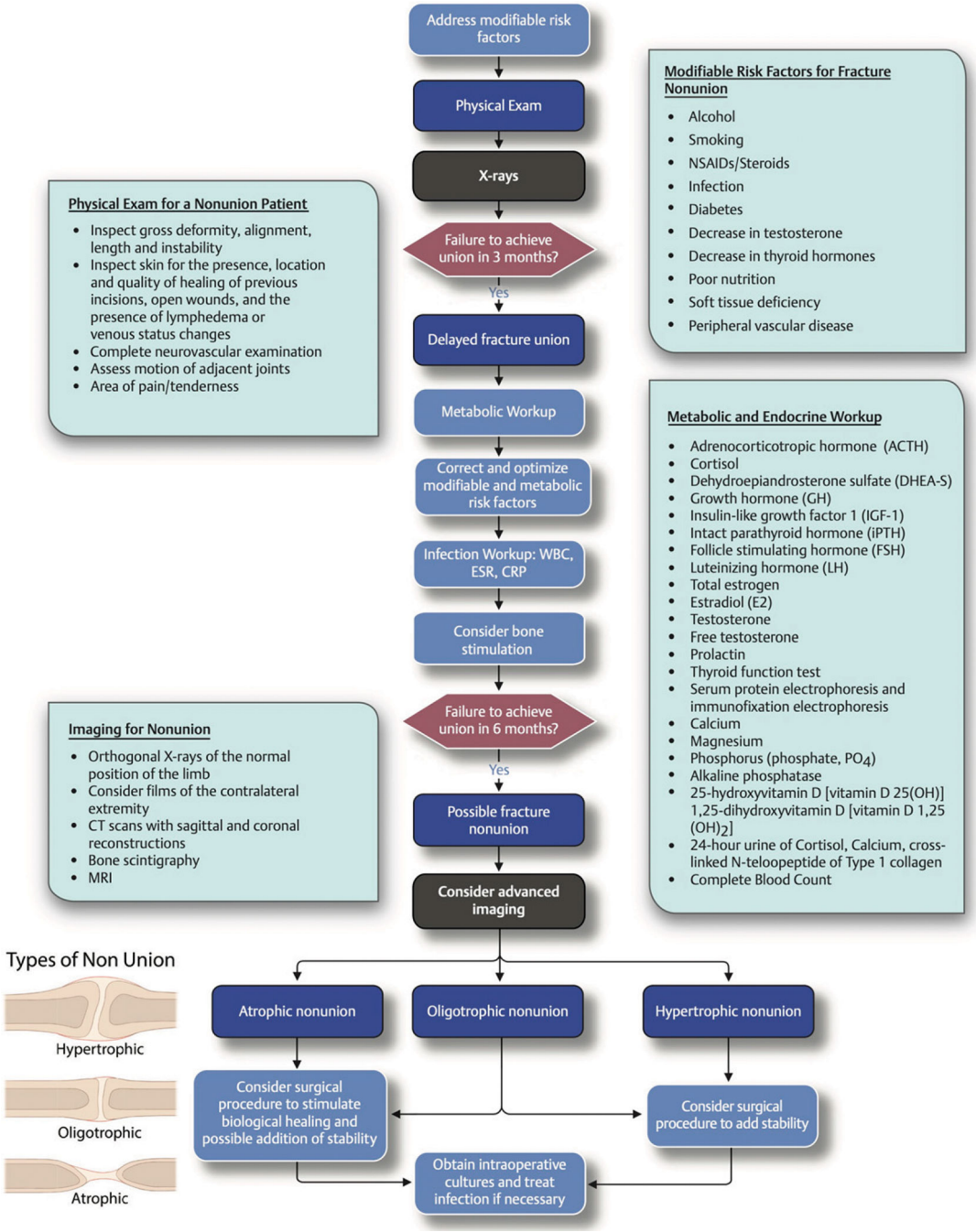

Fig. 5.

Modifiable and nonmodifiable risk factors that may lead to the development of nonunion. WBC = white blood-cell count, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein level, CT = computed tomography, MRI = magnetic resonance imaging, and NSAIDs = nonsteroidal anti-inflammatory drugs. (Reproduced, with modification, with permission of Thieme Publishers, from: Miclau T. Fracture delayed and nonunion. In: Marmor MT. Decision making in orthopaedic trauma. Thieme NY; 2017. p 154–5.)