The Biology of Bone and Ligament Healing

By

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Disclosure Statement: Dr. Arinzeh receives research funding from the Department of Defense, National Science Foundation, National Aeronautics and Space Administration, Musculoskeletal Transplant Foundation, New Jersey Commission on Spinal Cord Research, Christopher L. Moseley Foundation, and Integra Life Sciences, Inc. Dr. O'Connor is an owner of Accelalox Inc. which is developing novel therapies to promote bone regeneration, and receives research funding from the Musculoskeletal Transplant Foundation and the National Institutes of Health. Drs Cottrell and Turner have nothing to disclose.

Keywords:

- Bone Fracture Healing
- Tendon and Ligament Healing
- Arthrodesis
- Tissue Regeneration
- Wound Healing
- Charcot's Neuropathy
- Fifth Metatarsal Fractures

Key Points:

- Bone healing occurs through primary or secondary ossification to restore the functional integrity of the affected bone.
- Charcot's neuropathy and certain fifth metatarsal fractures have poor healing success rates that are exacerbated by specific risk factors and co-morbidities.
- Ligament and tendon healing is not a regenerative process but occurs through a distinct wounding healing process that requires short-term fibrocartilage formation and long-term tissue remodeling to restore function.

Synopsis

This review describes the normal healing process for bone, ligaments, and tendons including primary and secondary healing as well as bone to bone fusion. It depicts the important mediators and cell types involved in the inflammatory, reparative, and remodeling stages of each healing process. It also describes the main challenges for clinicians when trying to repair bone, ligament, and tendon with a specific emphasis on Charcot's neuropathy, fifth metatarsal fractures, arthrodesis, as well as tendon sheath and adhesions. Current treatment options and research areas are also reviewed.

A. Introduction

Foot and ankle function relies on bones, ligaments, and tendons (BLT) for strength, support, and movement. Injuries to these lower extremities occur frequently at work or during sport related activities and still account for over 20% of all emergency department visits annually.¹ Foot and ankle injuries are common in patients between 20-60 years old and frequently include ligament and bone. Trauma care has made advances over the past three decades which has contributed to a steady decline in the rate of ligament injury. Despite these improvements, the rate of serious bone injury has continued to grow over this same time.¹ BLT tissues injuries trigger an inflammatory response which stimulates synthesis of cytokines, growth factors, and other mediators to coordinate the normal tissue healing response. When BLT injuries are complex or associated with other co-morbidities and risk factors, mechanical or biochemical intervention is required to help return the injured tissue back to its original strength and function. The normal mechanisms that guide bone, ligament, and tendon healing will be a focus of this article as well as current treatment challenges for these injuries.

B. The Biology of Bone Healing

Fractured bone is capable of undergoing repair and regeneration. However, approximately 10% of fractures result in delayed fracture healing (delayed union), malunion or nonunion.² In these cases, the patients experience persistent pain and ultimately require medical intervention to promote healing of the fracture.^{3,4} Fracture healing can be classified into primary and secondary healing. Primary healing requires rigid fixation because it can only occur in the complete absence of motion at the fracture site while secondary healing requires minimization of motion (e.g. cast or splint) at the fracture site. Secondary healing benefits from limited motion at the fracture site to promote callus formation that will ultimately lead to internal immobilization of the fracture. The sequence of events that take place during fracture healing and bone development have been extensively studied at the cellular and molecular levels.⁵⁻⁹ These findings have increased our level of understanding of bone healing and may further advance surgical and therapeutic strategies for promoting the repair of damaged bone. This section will discuss the biology of different forms of fracture healing, with secondary healing discussed first since it is the most common form of healing, followed by an overview of bone-bone fusions.

Secondary Healing

Secondary healing involves endochondral ossification, which mediates the stabilization of the injury and restoration of damaged vasculature prior to regeneration of the tissue during the fracture healing process. This fracture healing process can be divided into three-overlapping phases: (1) inflammatory, (2) reparative, and (3) remodeling where intramembranous and endochondral ossification occur during the reparative phases (Figure 1).¹⁰ Various cellular components are recruited at different stages in response to growth factors and cytokines. The

inflammatory phase occurs immediately after injury. In humans the inflammatory phase lasts approximately 1 week whereas in mice, the inflammatory phase lasts less than 4 days.¹¹ The damaged vasculature and bone marrow facilitates the influx of primitive mesenchymal stem cells (MSCs) into the fracture site.¹⁰ During hemostasis, platelets release transforming growth factor- β (TGF- β) and platelet-derived growth factor (PDGF) for the stimulation and chemotaxis of undifferentiated MSCs and macrophages. Macrophages are initially recruited to remove debris, necrotic tissue and pathogens at the site of injury. A recent study found that the phenotype of callus macrophages changes during the progression of fracture healing and that macrophage depleted mice impairs fracture healing.¹² Macrophages express fibroblast growth factors 1 and 2 (FGF-1 and FGF-2), interleukin-1 (IL-1), and TGF- β during the inflammatory phase that may help promote angiogenesis within the fracture.¹² MSCs recruited from the exposed bone marrow, periosteum (outer lining) or endosteum (inner lining) differentiate into fibroblasts, chondrocytes, and osteoblasts during the reparative phase.¹³

Both, intramembranous (IM) and endochondral (EC) ossification occur simultaneously during the reparative phase. IM ossification primarily occurs at the peripheral ends of the presumptive callus as precursor cells differentiate into osteoblasts that lay down woven bone (“hard callus”). These osteoblasts express TGF- β , FGF-1 and FGF-2, insulin-like growth factor-I (IGF-I), and bone morphogenetic proteins (BMPs). In contrast, EC ossification requires the formation of cartilaginous intermediate tissue (“soft callus”) prior to ossification. Thus, EC occurs within the bulk of the fracture space where vasculature is initially limited. Within the “soft callus”, proliferating chondrocytes express TGF- β , IGF-I, IGF-II, BMP-2, BMP-4, and BMP-7 in association with increased collagen and cartilage matrix synthesis.¹⁴ Chondrocytes at the IM/EC interface (chondro-osseous junction) transition into hypertrophy while increasing

their cell volume as they continue to secrete cartilaginous extracellular matrix. Hypertrophy is initiated and regulated by expression of various transcription factors, primarily, *Runx2* and *Runx3*. As chondrocytes reach hypertrophy and begin to undergo non-apoptotic cell death, matrix metalloproteinases (MMP13 and MMP9) are released to degrade collagen type II and aggrecan (cartilage matrix components) while the hypertrophic chondrocytes express IGF-II and synthesize collagen X.¹⁴ Additionally, hypertrophy up-regulates the expression of vascular endothelial growth factor (VEGF). Cartilage is highly avascular, thus, the VEGF expression is crucial for vascular invasion necessary for ossification of the cartilage tissue. Along with new blood vessels, osteoclasts, osteoblasts, and bone marrow cells also invade the hypertrophic cartilage tissue from the IM/EC ossification interface. Osteoclasts partially resorb the cartilaginous matrix, while osteoblasts utilize any remaining matrix as a scaffold to deposit bone matrix. Eventually the matrix becomes mineralized, as bone cells begin to deposit hydroxyapatite (inorganic mineral present in bone).^{10,15} Mineralization is highly regulated by the expression of alkaline phosphatase, osteonectin (binds calcium), osteocalcin (hydroxyapatite regulator), TGF- β (increases alkaline phosphatase and osteonectin expression), and BMPs 2, 4, 7, and 9 (increase alkaline phosphatase and osteocalcin expression).⁸ IL-6 is produced by osteoblasts to stimulate bone resorption, while IGF-II modulates resorption by osteoclasts. Eventually woven bone replaces the mineralized callus, and the remodeling phase begins.^{2,10,15-17} Remodeling following fracture repair entails the replacement of woven bone with lamellar bone orchestrated by osteoprogenitor stem cells that differentiate into osteoblasts (bone-forming) and osteoclasts (bone-resorption). Osteopontin mediates anchoring of osteoclasts to bone matrix for resorption during remodeling.¹⁸ Overall, the remodeling phase occurs gradually in response to mechanical loading, and can last up to several years.

Primary Healing

Primary bone healing requires rigid fixation at both ends of the fracture to ensure that the fracture site is completely immobilized, which suppresses “callus” formation. In primary bone healing, cells do not form fibrous or cartilaginous tissue within the fracture gap. Instead, the fracture is repaired from woven bone laid down through intramembranous ossification. Two different types of primary bone healing exist, (1) gap healing and (2) contact healing.^{16,17} In gap healing, small fracture gaps are filled with woven bone, which is then remodeled to complete healing. During remodeling, osteoclasts create ‘cutting cones’, tunnel-like cavities through the bone and are trailed by osteoblasts that form new lamellar bone while reconstructing Haversian canals and osteons.^{19,20} Different from gap healing, contact healing requires the fracture fragments to be rigidly fixed in contact with each other (e.g., bone fusions). Since fracture gaps are absent, osteoclasts initiate the formation of ‘cutting cones’ and the reconstructions of the Haversian canals and osteons immediately, without the need for woven bone deposition. Primary healing requires rigid fixation while secondary healing benefits from some motion within the microenvironment of the fracture site.^{16,17,21}

Bone-Bone Fusions

Bone or joint fusions (arthrodesis) are performed surgically to relieve severe pain and restore stability at the joint (Figure 2).^{22,23} In bone-bone fusions, the surgeon completely removes the articular cartilage or fibrous tissue covering the end of the bones at the joint to fully expose the underlying bone tissue. These “exposed” surfaces are then compressed and held together by fixation with pins, screws, plates or rods at an optimal position to create the fusion, which will permanently limit the mobility at the joint.²⁴ Primary bone healing occurs where the bone surfaces are pressed into contact and held by rigid fixation. Osteoclasts and osteoblasts then

begin a primary healing response as discussed above.^{19,20} For example, the ankle is a very complex hinged synovial joint consisting of portions of these three bones: the talus, tibia and fibula; its anatomy allows for a wide range of motion (dorsi- and plantar-flexion, eversion and inversion). In ankle arthrodesis, the distal tibia is typically fused to the talus, and in some cases, the fibula is included in the fusion.²³ Viable candidates for arthrodesis include patients who suffer from developmental defects, post-traumatic osteoarthritis, rheumatoid arthritis, nonunion or poorly healed fractures, necrosis, implant/graft failure, or neuromuscular disorders (ex, post-polio myelitis). Commonly reported sites for arthrodesis procedures include the ankle,^{23,25-27} wrist,²⁸ foot,^{22,29,30} sacroiliac (rarely post-partum),³¹ and spine.³²⁻³⁴

Autogenous bone grafts are often utilized in arthrodesis, including ankle arthrodesis. Patients can benefit from osteoprogenitor cells within autogenous bone graft to augment the rate of bone fusion and reduce the risk of nonunion.²¹ Different from contact healing, the process of bone graft incorporation in arthrodesis models is similar to secondary fracture healing process where inflammatory, reparative, and remodeling phases are seen.²¹ Spinal arthrodesis with autogenous iliac crest bone graft remains the gold standard to relieve back pain associated with instability of the spine. Approximately 500,000 spinal arthrodesis surgeries occur per year in the United States.³³ In those joints in which immobility causes severe disability such as the hip, cubitus (elbow) and knee joints, arthrodesis is considered only in selected patients who are not suitable candidates for preferred operative treatments (e.g., total joint replacement) or after non-operative and preferred operative treatments have failed.^{35,36} In the latter, arthrodesis is generally a salvage procedure.

Complications associated with arthrodesis procedures have been reported and include a 10-60% rate of adjacent joint deterioration in the long-term, 10-20% rate of nonunion, and 3-

25% rate of post-operative infection.²⁵ Joint replacement (arthroplasty) has become increasingly popular over arthrodesis because it preserves mobility of the joint, diminishes pain significantly and may decrease the risk of arthritis on the neighboring joints from elevated stress as increased stress in neighboring joints is a drawback of arthrodesis.^{26,37} Evidence shows that in 2004, ankle arthrodesis was performed 9.8 more times than total ankle arthroplasty (TAA) and by 2009 ankle arthrodesis was performed 2.3 times more than TAA.²⁶ A 4 year follow-up study comparing ankle arthrodesis to TAA found that patients who underwent ankle arthrodesis reported ankle osteoarthritis scores (AOS) for pain as 51.2 and for disability as 44.5, while patients post-TAA reported AOS for pain as 26.0 and for disability as 33.2. An AOS score of 0.0 indicates no pain or disability. Although TAA surgery resulted in significant pain relief, 40.5% of the patients within the TAA group required post-operative surgical intervention during the follow-up period compared to 21.7% in the arthrodesis group of patients.²⁵ Another report found that adjacent joints become arthritic after arthroplasty more than after arthrodesis during a 5-year follow up.²⁷ However, in 2014 the American Orthopaedic Foot & Ankle Society (AOFAS) endorsed TAA surgery as an established operative treatment option for patients with arthritic conditions affecting the ankle joint.³⁸ Total joint replacement is not an option for everyone, certain criteria such as young age, high activity, obesity, misalignment, and history of alcohol and drug consumption are considered risk factors for joint replacement surgery as they may lead to premature wear of implants and require several revision surgeries.^{25,27} Overall, both arthrodesis and arthroplasty are widely used today to treat end-stage arthritic joints, and the selected route of operative treatment is specific to the patient depending on health risk factors, clinical history of the patient, the degree of damage, and the specific joint in need of repair.

C. Clinical Challenges in Bone Healing

Bone, ligaments, and tendons provide important functions to the foot and ankle. When these tissues are injured, inflammation occurs followed by a highly ordered and complex process that promotes healing. Many BLT injuries such as ankle sprains and osteochondral lesions (OCLs) can heal within 6-8 weeks with non-operative measures such as immobilization or reduction in strenuous activity. However, impaired BLT healing is frequently encountered in both inpatient and outpatient situations. Impaired BLT healing is associated with risk factors such as extreme of age, estrogen deficiency, smoking, osteoporosis, diabetes mellitus, alcohol abuse, obesity, non-compliance, chronic site infection, deformity, and neuropathy. Impairment of BLT healing can have devastating consequences to the patient and may lead to injury related morbidity and mortality. Poor fifth metatarsal healing and Charcot neuropathic osteoarthropathy (CN) are both clinically challenging conditions that result from impaired BLT healing. Arthrodesis is a common procedure used to resolve many foot and ankle conditions like CN. However, complications with this procedure are frequent in populations with comorbidities and risk factors. The pathogenesis, etiology, and current treatment challenges for fifth metatarsal and Charcot neuropathic osteoarthropathy will be reviewed below.

Fifth Metatarsal Fractures: Epidemiology, Pathogenesis, and Diagnosis

Stress and damage induced by direct and indirect injuries to the bone are typically healed by the natural bone remodeling process. Direct damage such as high-impact trauma, including motor vehicle accidents or heavy falling objects, can apply a direct force and lead to metatarsal fractures at any location.³⁹ In contrast, indirect forces applying torque to the foot can result in fractures of the metatarsal shafts.⁴⁰ Repetitious stress or chronic damage of a metatarsal can overwhelm the normal remodeling process and weaken spots in the bone, leading to the

development of stress fractures. Metatarsal fractures account for 35% of all foot fractures and approximately 25% result from stress fractures of the fifth metatarsal.⁴¹⁻⁴³ Unlike normal fractures, stress fractures do not heal by callus formation instead they undergo direct remodeling.^{44,45} Direct remodeling to heal stress fractures is slow and often stress fractures progress to complete fractures which resist healing and often leads to nonunion.^{44,45} Patients with metatarsal fractures typically show signs of antalgic gait, dorsal edema, and ecchymosis. Clinical, neurovascular, physical, and radiographic evaluations are used to diagnose metatarsal fractures.⁴⁶

Fifth Metatarsal Fractures: Anatomical Considerations

Of the metatarsals, fifth metatarsal fractures are the most common. They account for 68% of metatarsal fractures in populations between 20-50 years old.^{47,48} The fifth metatarsal consists of a base, neck, head, the tuberosity, and the diaphysis (shaft). The lateral band of plantar fascia, the peroneus brevis tendon and the peroneus teritus tendon are all soft tissues associated with the fifth metatarsal.^{49,50} The osteology of the fifth metatarsal is curved and the dorsal-plantar cortex is thinner than the medial-lateral cortex. Blood supply around the fifth metatarsal varies. The tuberosity's blood supply comes from multiple metaphyseal vessels and multiple branches of the nutrient artery. The proximal diaphysis, however, receives its blood supply solely from the nutrient artery.^{51,52} Fracture in the proximal diaphysis is more likely to disrupt the blood supply and contributes to high rates of nonunion, delayed union, or re-fracture.⁵³ Proximal fifth metatarsal fractures are sometimes classified into three anatomical zones. Zone I is the most proximal and includes the tuberosity. Zone II is the meta-diaphyseal junction and includes the eponym "Jones Fracture" region. Zone III occurs at the proximal diaphysis. Fractures occurring

in zone I and zone II usually occur from direct and indirect acute damage while fractures in zone III result from pathological stress.⁵⁴

Fifth Metatarsal Fractures: Treatment and Challenges

For treatment and prognoses reasons, fractures in the fifth metatarsal are classified into types I, II, and III.⁵⁰ Type I fractures involve the tip of tuberosity and have been associated with sport related injuries. These tuberosity avulsion fractures occur during inversions of the foot and ankle while they are in plantar flexion. Studies have shown that both the lateral band of plantar fascia and the peroneus brevis are involved.^{39,55} Type I injuries are usually completely healed within 6 to 8 weeks if the avulsion fracture is nondisplaced. Conservative treatment includes rest, ice, elevation above heart-level, and often a soft Jones dressing with stiff soled shoe, non-weight bearing short leg cast, or removable cam walker. Complications from type I fractures are unusual, although Bigsby et al. recently demonstrated in a prospective study that 25 percent of patients have pain a year after the injury.⁵⁶ This discomfort is typically correlated with displaced fractures that may lead to nonunion. If a nonunion persists some clinicians use pulsed electromagnetic field therapy or external bone stimulators to promote healing. Pulsed electromagnetic field therapy has been effectively used in the management of delayed unions and nonunions of type I fractures while little research has been conducted on the efficacy of other bone stimulators.^{40,56,57}

In type I fractures, surgical intervention is necessary if displacement is greater than 3 mm, a step-off of more than 1 to 2 mm on the articular surface with the cuboid, 30% of the metatarsal cuboid joint articulation is involved, or if callus formation is not evident by the eighth week after radiographic examination.^{39,58,59} Surgical interventions for fifth metatarsal fractures vary but include screw fixation, tension banding, and percutaneous pinning.^{42,55,60-66} In one study,

the effectiveness of percutaneous biocortical screw fixation for displaced intraarticular zone I proximal fifth metatarsals was studied.⁶⁴ The data demonstrated that percutaneous biocortical screw fixation effectively decreased preoperative displacement and allowed for complete healing within 4 to 6 weeks. Biocortical fixation was suggested to be superior to intramedullary fixation because it disperses the load over a greater cortex surface area and offers greater stability.

Type II injuries occur within the junction of the metaphysis and diaphysis and generally result from an acute sports injury.⁶⁷ Unlike type I injuries, type II injuries are not caused by inversion. These fractures are thought to develop when a significant adduction force is applied to the forefoot while the ankle is in plantar flexion.⁵⁹ Stress fractures can also develop in this area and need to be successfully distinguished from an acute injury in order to develop a proper treatment plan for the patient. Clinical presentation, patient history, and radiographic findings need to be carefully examined to differentiate between acute and stress fractures. Initial treatment requires immobilization in a posterior splint, strict non-weight bearing, icing, and elevation of the limb. For most non-displaced, acute fractures conservative treatment is recommended.^{39,59,67}

However, acute type II diaphyseal fractures pose several challenges for successful healing. Since it is difficult to distinguish between acute and stress fractures, incorrect diagnosis often leads to improper treatment and results in a high incidence of nonunion. Therefore, it is important to gain a thorough patient history and carefully examine all radiographs to properly diagnose the patient. Also, treatment of acute diaphyseal fractures requires casting and strict non-weight bearing for long periods of time so many patients are non-compliant. Even with proper diagnosis and treatment many acute diaphyseal fractures result in nonunion or re-fracture.⁵⁹ In a study of basketball players carefully diagnosed with acute proximal diaphysis injury, 50% percent were found unhealed after 12 weeks of conservative treatment.⁶⁸ Impaired vascular

supply and premature weight bearing are thought to cause poor healing in these injuries.^{52,53,67} A 2005 study, compared conservative and surgical treatment of these fractures.⁶⁹ In this study, approximately 50% of the patients were treated with intramedullary screw fixation followed by immobilization for a period of two weeks. The remaining 50% of the patients were immobilized in a non-weight bearing, short leg cast for eight weeks, followed by a walking cast, until there was radiographic and clinical evidence on fracture union. Forty-four percent of the conservatively treated group were found to have a visible symptomatic fracture 26 weeks post-fracture while only 5% of the surgically treated group did. Two other articles which reviewed 32 studies showed that surgical intervention generally reduced the risk of nonunion.^{70,71} As a result of these challenges, early surgical fixation and prolonged use of a customized weight-bearing orthosis are reasonable options for treatments.^{67,72}

Type III injuries include stress fractures of the fifth metatarsal and result from chronic and repetitive microtrauma. Type III fractures occur less frequently than type I and II fractures, however they have a much higher propensity to develop into a delayed union, nonunion, or re-fracture.^{59,73} Radiographically they are distinct from acute fractures and have been classified by Torg based on severity.⁷⁴ An early stress fracture displays cortical thickening (Torg I) while older stress fractures have a thicker fracture line (Torg II) or absence of the medullary canal (Torg III). Most type III injuries require surgical fixation (Torg II & III) instead of conservative treatment (Torg I).⁷⁵ Conservative treatment is advised only for patients with early stress fractures that would like to avoid surgery and do not mind long-term immobilization. Surgical fixation, bone stimulators, bone grafts, and other therapies have been used to manage problematic stress fractures and are similar to the options already discussed for acute metatarsal injuries. Surgical treatment of these fractures has similar challenges and healing outcomes as

described for type II metatarsal fractures. However, operative treatment still results in prolonged immobilization time.^{42,54,63-65} Current research is focused on improving the surgical methods to reduce the time a patient is prohibited from activities. One study using teriparatide an FDA approved bone stimulator for osteoporosis treatment focused on improving healing outcomes without surgical intervention.⁴² Improvement was seen in two patients with metatarsal stress fracture healing after treatment with teriparatide, vitamin D3, and calcium citrate. However the scope of this study was limited and future studies are warranted. A future study aims to test the efficacy of adding bone marrow stem cells to the surgical site in order to promote better healing outcomes for metatarsal stress fractures.⁶⁵ Continued research on these specific injuries is warranted and could result in improved healing success in patients with type III fractures as well as those suffering from type I and II fractures.

Charcot Neuropathic Osteoarthropathy (CN): Epidemiology, Pathogenesis, and Diagnosis

Charcot neuropathic osteoarthropathy (CN), commonly known as Charcot's foot, is a condition where acute localized inflammation affects the bones, joints, and soft tissues of the foot and ankle. CN results from various peripheral neuropathies but is commonly associated with diabetic neuropathy. The precise incidence of CN is unclear but it was recently estimated to effect 0.08%-0.13% of the population but has a higher incidence in the diabetic community (7.5-9%).⁷⁶⁻⁷⁸ CN can be classified in terms of clinical stage, anatomical localization, and the stage of natural history which have been described extensively in the literature.⁷⁸⁻⁸¹ Proper and prompt diagnosis of CN is important to minimize the risk of osteophyte development, ulceration, infection, deformity, and amputation. Recent studies demonstrate that plain radiographs are insufficient at diagnosing Charcot foot injuries.^{9,82,83} Many studies have shown that advanced imaging such as magnetic resonance images (MRIs) are better at properly diagnosing CN.^{9,83-90}

CN's cause is thought to be multi-factorial resulting from several components such as diabetes, sensory-motor neuropathy, or metabolic abnormalities of the bone. These components contribute to unresolved acute inflammation in a specific ankle or foot region which inflicts varying degrees of bone damage, subluxation, dislocation, and deformity. Midfoot collapse, commonly known as "rocker-bottom" foot, is a classic deformity associated with CN. The pathogenesis or process by which CN progresses is not well understood, however, two theories have been described.⁷⁸ The neurovascular theory states that an autonomic stimulated vascular reflex causes hyperemia and increased bone resorption which leads to joint destruction and trauma. The neurotraumatic theory suggests that CN results from peripheral neuropathy which causes sensory loss in the feet. As a result, repetitive strain, pain, and overload are unrecognized in the insensate region which eventually leads to unknown trauma or injury that triggers the development of CN.^{78,91-94}

Charcot Neuropathic Osteoarthropathy (CN): Anatomical Considerations

CN's classification systems have varied over the years, for simplicity this review will use the classical model to describe the stages of this condition. Stage I is the acute phase and is characterized by fragmentation. The midfoot is usually affected and is characterized by swelling and warmth. Some radiographs display osteopenia, periarticular fragmentation, and subluxation of joints but most will seem typically normal. The acute stage is often misdiagnosed as gout, deep vein thrombosis, or soft tissue injury and as a result the disease can progress to Stage II or III.⁹⁵⁻⁹⁸ Stage II represents the reparative phase where edema and warmth decrease, bone absorption occurs and bone formation occurs. Stage III is known as the chronic or inactive stage.^{78-80,99} Many of the acute signs such as warmth and redness disappear, but stable

deformities may develop in the third stage.^{78-80,99} Common deformities to develop include collapse of the plantar arch in the midfoot or a prominent medial aspect of the midfoot. These deformities often result in formation of abnormally high-pressure areas on the foot that become particularly prone to ulceration.^{78-80,99}

Charcot Neuropathic Osteoarthropathy (CN): Treatment and Challenges

It is of utmost importance that CN be properly diagnosed and treated during the acute stage to prevent further progression of the condition. Conservative treatment is typical in stage I and includes immobilization with a non-removable device such as a total contact cast (TCC) with instructions to be non-weight bearing. Literature exists that suggests that weight-bearing while immobilized does not negatively affect the resolution of acute CN.^{9,82,83,89,98,100,101} The duration of immobilization is dependent upon the extremity and radiographic evidence of ossification at the site but on average it can range between 8-52 weeks.^{77,82,84,87,89,95,98} Conservative treatment however has its challenges. Prolonged casting and immobilization can lead to complications such as ulceration and patient compliance during prolonged immobilization periods is not easy to guarantee.^{97,101-104} Other orthotic devices are available (removable walking boots, patellar tendon brace) that help increase patient compliance though studies indicate that use of the orthotic devices increases the length of time patients are immobile.^{98,105,106} Surgical intervention during this acute stage has some major risks for wound healing complications and infection.^{87,98,99,103,107,108} Additionally, bone quality is a main concern because CN is characterized by the pathogenic process of osteoclastic resorption and fragmentation. The bone quality is feared to be suboptimal for proper fixation which could lead to hardware failure, pseudoarthritis, delayed union, or non-unions.^{98,99,108} Therefore, surgery in the acute phase of CN is restricted to patients with severe dislocation or instability, failure of conservative treatment,

poor vascular supply near the affected limb, patient's with co morbidities, or evidence of an anatomic location breakdown. Early surgical intervention during acute CN in diabetic patients was found to be effective at increasing arthrodesis without complications.¹⁰⁸ Stage II is considered a better time for surgical intervention. Surgical intervention is typically completed with an open reduction and internal fixation.⁸² If the process has reached the stage III, arthrodesis is typically more difficult to achieve because an osteotomy needs to be conducted and may result in ulcerations.^{82,97,102,109}

Surgical intervention for CN includes non-locking and locking plate and screw fixation, intramedullary screw or nail fixation, external fixation, internal fixation or a combination of these methods.¹¹⁰⁻¹¹² Surgical method choice is typically dependent upon bone quality. If bone quality is poor, internal fixation may use a superconstruct.^{113,114} that connects affected joints to non-affected joints in order to gain stability. However, this method requires large incisions which increases the risk of infection, soft tissue complications, and disruption to the osseous vascular supply.¹¹⁰ Intramedullary screw and nail fixation requires a smaller open approach and thus limits the risks associated with a large incision. Intramedullary fixation also gives the clinician the ability to correct and realign deformed bone with use of guide wires but complications resulting from hardware failure or migration can occur.^{5,110,113-119} External fixation is used with or without internal fixation if a patient is suffering from severe deformity or instability. Most external devices use tensioned wires to stabilize multiple planes and promote inter-fragmentary compression to prevent implant loosening, neutralize stress placed on the foot, and assist in deformity correction.^{112,120,121} Complications resulting from external fixation are more common in diabetic patients and include pin loosening, pin breakage, pin failure, stress fractures, osteomyelitis, and pin tract infections.¹²²

If surgical correction requires arthrodesis, the use of bone graft and/or adjuvants may be necessary. Autogenic bone is typically preferred but allogenic, synthetic, or combinatorial bone graft substitutes can be used. Autogenic bone graft is typically harvested from the iliac crest, distal tibia, or fibula and is used to fill osseous voids.¹²³⁻¹²⁶ Disadvantages of autogenic bone use are donor site morbidity, pain, and availability.¹²³ Allogenic grafts avoid the disadvantages associated with autogenic bones and reduces the operating time needed to obtain the bone. Other bone graft substitutes such as demineralized bone matrix (DBM), calcium sulfates, and hydroxyapatites are also readily available as alternatives to allogenic grafts. Incorporating orthobiologics, such as bone morphogenetic protein (BMP), bone marrow aspirate, or platelet-rich plasma, into the wound or osseous region can enhance healing.^{82,127,128} In a recent study, patients at high risk for surgical failure (immunodeficient, multiple comorbidities, recurrent infections) underwent CN surgery for deformity correction using allogenic bone graft supplemented with platelet-rich plasma.¹²⁹ Arthrodesis was achieved in 90% of the patients and that were also infection free at 26 months. Similar favorable outcomes have been achieved with adjunctive treatments such as bisphosphonates,¹⁰⁶ intranasal calcitonin,¹³⁰ or bone stimulation.⁵⁷

Since CN can be challenging to diagnosis and treat, it is recommended that advanced imaging be conducted on any patient with peripheral neuropathy, pain and swelling to rule out acute CN. Immobilization appears to be the preferred treatment for acute stage CN even though some evidence suggest surgery at this stage can improve outcomes. When surgical intervention is necessary, use of orthobiologics or adjuvants appears to increase positive healing outcomes.

Arthrodesis of the Foot & Ankle

Fusion of the foot or ankle is typically used as an end-stage procedure to salvage the limb. Patients with certain risk factors such as smoking, diabetes, alcohol abuse, obesity or

genetic disorders are at greater risk for complications associated with fusion. Surgical techniques to promote fusion are numerous and include use of internal and external fixation. Examples of these methods were discussed above with regards to treating stage II and III Charcot's foot. Complications are common with arthrodesis and include hardware failure, pin migration, infection, delayed-union, nonunion, stress fractures, or cortical hypertrophy. Biological augmentation with osteoconductive or osteoinductive reagents have been the focus of research to increase fusion success. Some studies show promising results with use of osteobiologics such as rhBMP-2 to promote arthrodesis.^{23,131,132} while others note complications such as heterotopic ossification.^{23,133,134} For an extensive review of the challenges involved in arthrodesis, please see Rabinovich et al. and Slater et al. on complex ankle arthrodesis.^{23,135}

D. Biology of Ligament and Tendon Healing

Ligament and Tendon Healing

Tendon and ligament ruptures and tears are common injuries. Acute tendon and ligament injuries are often caused by extrinsic traumatic injuries.¹³⁶ However, rapid acceleration-deceleration movements have been associated with acute sports-related tendon injuries.¹³⁷ Chronic degenerative changes in tendons and ligaments also can lead to susceptibility for rupture or tear.¹³⁸ Possible mechanisms leading to chronic degenerative changes in tendon include damage from reactive oxygen species produced in conjunction with load-induced ischemia-reperfusion.¹³⁹, strain induced cyclooxygenase expression leading to PGE₂ synthesis, inflammation, and matrix metalloproteinase synthesis and accumulated small partial tears that would weaken the overall tensile strength of the tendon.¹⁴⁰⁻¹⁴³ Use of fluoroquinolone antibiotics, such as ciprofloxacin, also has been associated with increased risk of tendon rupture.^{144,145} Cell

based studies have shown that ciprofloxacin can induce IL-1 β expression that could possibly trigger inflammation-related tendon damage,¹⁴⁶ though others have suggested that IL-1 β may not be involved in tendinopathy.¹⁴⁷

Treating tendon and ligament injuries is complicated by an incomplete understanding of how tendons and ligaments heal. For instance, there is significant variation in the ability of some ligaments to heal since knee medial collateral ligament injuries are often managed non-surgically during healing while knee anterior cruciate ligament injuries generally undergo surgical repair.¹⁴⁸⁻¹⁵⁰ The location of the injury may also affect treatment and outcomes, such as management of adhesion formation in injuries involving the tendon sheath or injuries involving the muscle-tendon (myotendinous) junction or the bone-tendon interface (enthesis).

Unlike bone, tendons and ligaments do not heal by tissue regeneration but rather through a typical wound healing process in which the defect is filled with fibrous tissue. Complete or partial disruption of the tendon or ligament leads to a predictable healing pathway that includes an early phase, reparative phase, and a remodeling phase that increases repaired tendon tensile properties. The early phase consists of hematoma formation, inflammation, and initiation of cell proliferation at the injury site. During the reparative phase, proliferating tenocytes from the tendon and epitenon and proliferating cells from surrounding tissues, e.g., the tendon sheath, localize at the injury site and begin to heal the rupture by formation of fibrous tissue that contains a high proportion of type III collagen. This reparative phase can last for weeks during which time the healing tendon remains mechanically weak and subject to re-injury. During the remodeling phase, the fibrous repair tissue becomes less cellular, glycosaminoglycan concentrations decrease, and the proportion of type I collagen increases. Eventually the collagen fibers and tenocytes align with the direction of stress to increase the repaired tendon tensile

strength. The remodeling phase can last several months. Factors that mediate inflammation, angiogenesis, cell proliferation, and extracellular matrix remodeling appear to be involved in the different phases of healing based upon gene expression studies. However, the mechanisms that coordinate these gene expression events and the necessity of each factor in tendon healing is not well understood.

Recent studies have cataloged patterns of gene expression following tendon or ligament injuries. Using a mouse flexor tendon defect model, Juneja et al. found a rapid increase in type III collagen expression that peaked between 2 and 3 weeks after surgery.¹⁵¹ Interestingly, expression of anti-inflammatory TGF- β_3 increased approximately 6-fold between 7 and 14 days after fracture which would coincide with the transition from the early to reparative phase of healing. Manning et al. found that type III and type I collagen expression was reduced more than 10-fold immediately after canine flexor tendon transection and repair as compared to normal tendon, while inflammatory related gene expression (IL-1 β and COX-2) was elevated.¹⁵² Manning et al. also observed that after 9 days, type III collagen gene expression had increased suggesting initiation of the reparative phase. More recent gene expression studies have been conducted using human tendon samples collected during surgery. Using microarrays, Brophy et al. analyzed gene expression between anterior-cruciate ligaments that had been torn <3 months (acute), between 3 and 12 months (intermediate), or >12 months (chronic).¹⁵³ Ends of the torn ligaments were collected during surgical repair and so no intact specimens were available for comparison. Still, the analysis found that genes involved in extracellular matrix reorganization were expressed in the acute samples, while the chronic samples showed reductions in collagen gene expression and an interesting >30-fold decline in periostin expression. Chaudhury et al. compared rotator cuff tendons that had been symptomatic for at least 1 year with small (<3 cm)

and large (>3 cm) tears to control rotator cuff tendons using microarrays.¹⁵⁴ The results confirmed many previous studies, such as significant changes in extracellular matrix remodeling gene expression. Perhaps more important, analysis of the microarray data showed clear demarcations in gene expression patterns between the 3 different groups, indicating significant differences in the injury response between small and large tears.

The Tendon Sheath and Adhesions

A complication associated with tendon injuries is formation of adhesions between the tendon and the surrounding sheath or other tissues that limits range of motion.^{155,156} Cellular continuity between the tendon and surrounding tissue often occurs following injury as cells proliferate from the tendon and surrounding tissue to form the initial fibrous repair tissue. However, these cellular continuities must be resolved to allow the tendon to move freely and without limiting the range of motion. Nominally, these cellular continuities are resolved during healing and can be aided by early movement of the healing tendon and by non-steroidal anti-inflammatory drug treatment.^{157,158} Failure to resolve the cellular continuities appears to lead to adhesion formation. Given the nature of the adhesions, the pathological mechanism leading to adhesion formation could be failure to slow cell proliferation during the repair phase, failure to stop production of predominant type III collagen fibrous tissue, failure to produce sufficient levels of extracellular matrix remodeling enzymes, or a combination of these processes.¹⁵¹ Additional research is needed to understand the etiology of adhesion formation and for improving methods to prevent adhesion formation.

Healing at the Enthesis and Myotendinous Junction

Tendons and ligaments are connected to bone through a specialized fibrocartilage and calcified fibrocartilage structure called the enthesis.¹⁵⁹⁻¹⁶² Tendons connect to muscle at

myotendinous junctions in which the tendon collagen fibrils interact with the sarcolemma of the muscle to allow force transmission.¹⁶³ Despite the efficiency with which mechanical forces are transmitted through the enthesis and myotendinous junction, these junctions are mechanical weak points and sites of degenerative changes. Ruptures at myotendinous junctions appear to heal well and are often treated non-surgically.¹⁶⁴ In contrast, healing of ruptured entheses is more problematic and typically the normal structure of the enthesis is not rejuvenated. Instead, in the repaired tendon-bone junction, the tendon and bone are joined by fibrocartilage.^{165,166}

Current Research Areas

There is yet no gold-standard animal model for studying tendon repair, which makes comparison of gene expression and other data between models and species difficult. Complete and partial transection of the mouse Achilles tendon may be the best model to study the basic molecular and cellular events associated with tendon healing. The mouse Achilles tendon model allows for partial or complete transection, for no or simple suture repair, as well as testing of biomaterials and grafts. The mouse allows for use of genetically modified animals to directly assess gene functions on tendon healing, and healing can be measured using molecular, histological, and mechanical methods.¹⁶⁷⁻¹⁷³ However, any conclusion drawn from mouse Achilles tendon studies would need to be confirmed using clinical samples as there are clear differences in healing potentials between different tendons and species.

Research to improve tendon and ligament healing, improve healing at the enthesis or myotendinous junction, and that will prevent or minimize adhesions is ongoing.^{136,174,175} Efforts are focused on manipulating inflammation,^{158,176-180} using growth factors to control cell fates,^{33,181} altering extracellular matrix reorganization,^{182,183} and development of grafting materials.¹⁸⁴ Another concept being explored is to recreate a healing environment that

recapitulates that which occurs during fetal development in order to promote scarless wound healing.¹⁸⁵

E. Summary

Multiple factors such as genetic predisposition, comorbidities, anatomy, proper and prompt diagnosis, treatment type (conservative or surgical), and patient compliance all contribute to the challenges associated with effectively treating fifth metatarsal injury, Charcot neuropathic osteoarthropathy, and arthrodesis. Even though these conditions have been extensively studied, successfully healing these conditions is still difficult. Research suggests that effective treatment begins with rapid and accurate diagnosis of the condition which is best completed using advancing imaging such as MRI. The stage of the condition, patient risk factors, and comorbidities dictate whether conservative treatment or surgical intervention is necessary. Conservative treatment often leads to re-fracture, nonunion, or secondary complications like ulceration or infection. Failed conservative treatment eventually leads to surgical intervention. Surgical intervention can include internal fixation, external fixation, bone grafts, orthobiologics, adjuvants, or a combination of these methods, which each have their drawbacks including pin migration, heterotopic ossification, or hardware failure. Despite these disadvantages, conservative, and surgical treatment can be successful. However, additional randomized controlled trials applying new or improved methods needs to be conducted in order to justify their use on bone, ligament, and tendon related injuries in the future.

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FIGURE LEGENDS

Figure 1. Representation of fracture repair including growth factors and cytokines at each stage.

From Sfeir C, Ho, L., Doll, B. A., Azari, K. and Hollinger, J.O. Fracture Repair. In: Lieberman JRaF, G. E., ed. *Bone Regeneration and Repair*. Totowa, NJ: Humana Press Inc.; 2005:21-44, with permission.

Figure 2. Hallux valgus deformity (a) corrected with (b) arthrodesis of tarsometatarsal joints (Lapidus procedure). From Gougoulas N, Lampridis V. Midfoot arthrodesis. *Foot Ankle Surg*. 2016;22(1):17-25, with permission.

Inflammatory Phase- Vascular endothelial damage results in hematoma formation, complement cascade, and clotting cascade leading to the accumulation of PMNs, lymphocytes, platelets, blood monocytes, macrophages, neutrophils, osteoclasts, and undifferentiated cells. The cells express various genes including TGF- β , FGF-I, FGF-II, PDGF, IGF-I, IGF-II, BMP-2,-4,-7, osteonectin, BMPR I/II, IL-1, IL-6, and GM-CSF.

Reparative Phase (Fibrocartilage Callus Formation)- Fibrous tissue and new cartilage are beginning to form and revascularization is taking place. The appearance of macrophages, chondroblasts, chondrocytes, osteoclasts, fibroblasts, and endothelial cells is seen along with the expression of TGF- β , FGF-1, FGF-2, PDGF, IGF-I, IGF-II, BMP-2,-4,-7, osteonectin, fibronectin, BMPR I/II, smads 2,3,4, IL-1, IL-6, and collagens (II,III,IV,V,VI,IX,X).

Reparative Phase (Bony Callus Formation)- Intramembranous and endochondral ossification is taking place to lay down new woven bone. The appearance of macrophages, chondroblasts, chondrocytes, osteoblasts, osteoclasts, and endothelial cells is seen along with the expression of TGF- β , FGF-1, FGF-2, PDGF, IGF-I, IGF-II, BMP-2,-4,-7, osteonectin, osteopontin, osteocalcin, BMPR I/II, smads 2,3,4, IL-1, IL-6, and collagens (I, II, III, IV, V, VI, IX, X).

Remodeling Phase- Replacement of woven bone by lamellar bone and the resorption of excess callus. Gradual modification of the fracture region leads to restoration of normal bone architecture.

a

R

b