Fracture Healing and Progress Towards Successful Repair

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Abstract Despite the intrinsic healing capacity of bone and advancements in orthopedic technologies, well-established interventions, including autologous bone grafting, have had a relatively limited impact on easing the burden of a proportion of the 5–20% of long bone fracture patients who suffer from delayed healing or nonunion. In this chapter, we describe how the biology of bone development and bone homeostasis are recapitulated in bone healing, and how immunological and mechanical factors regulate healing. We present the current barriers faced clinically, outlining some of the main risk factors associated with the development of delayed healing and nonunion, with a focus on bone infection, and how it hijacks the bone healing process, ultimately leading to bone destruction. We conclude by depicting the outlook on fracture healing, outlining the progress to-date and the biggest challenges we face, while highlighting how our increasing understanding of the immunomodulation of bone healing can potentially be harnessed to develop innovative strategies for patient benefit.

Keywords Fracture healing · Mechanical factor · Immunological factor · Delayed healing · Nonunion · Risk factor

Introduction

Bone is a dynamic and highly vascularized tissue, which has the rare capacity to heal without the formation of a fibrotic scar [1]. Advancements in orthopedic technologies and methods of fracture fixation have led to high standards in the treatment and care of patients with fractures [2]. However, despite its intrinsic healing capacity and modern orthopedic fixation methods, a proportion of fractures exhibit delayed healing or result in nonunion. In the case of large bone defects, interventions such as

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bone grafting are used to replace damaged and lost bone tissue, which remains the second most transplanted tissue after blood [2] with over 2.5 million bone grafting procedures taking place worldwide annually [3]. Complications including disturbed vascularization, soft-tissue damage, lack of adequate mechanical stability, and bacterial infections have all been identified as likely causative factors for impaired healing, although their specific contributions remain to be adequately addressed, while the observed rates of delayed healing or nonunion (10–20% of all cases) highlight that it remains a major clinical problem [4, 5]. Autologous bone grafting remains the clinical gold standard for the treatment of complex long bone defects despite the known constraints of donor site morbidity, limited tissue availability, and a reduction in the regenerative capacity of donor tissue with increasing donor age [3, 6–8]. Grafting alternatives have emerged in the form of tissue-engineered osteoinductive and osteoconductive biomaterials, paving the way for combinatorial treatment strategies that utilize modern methods of fracture fixation together with biomaterials to support bone healing. Furthermore, the crosstalk between immune cells and the biology of bone healing is now better understood than ever before [9]. Combining these developments may enable innovative solutions in the form of "immuno-informed" tissue-engineered biomaterials and fracture fixation technologies, which might elicit favorable immune responses upon implantation and thereby complement the intrinsic healing capacity of bone. In this chapter, we review our understanding of the process of fracture healing with a focus on the role of immunology, outlining our progress towards overcoming the barriers towards successful repair, which are currently faced clinically.

Origin of Bone

The processes of embryonic bone development and bone homeostasis in the adult are recapitulated, at least in part, in the process of bone healing after fracture. The intrinsic healing capacity of bone has evolved in parallel with the functionality of bone tissue [10]. Bone mechanically supports soft tissue, is a lever for the action of muscles, protects the central nervous system from trauma, regulates calcium levels in extracellular fluid, and houses and supports hematopoiesis [11]. Bone begins to form during the sixth to seventh week of embryonic development via two osteogenic pathways, namely intramembranous ossification, which gives rise to the flat bones of the cranial vault, including the cranial suture lines, some facial bones, and parts of the clavicle and mandible, and endochondral ossification, which gives rise to long bones and bones at the base of the skull [12, 13].

During early limb development, endochondral ossification (Fig. 1) is initiated at the limb bud with the condensation of mesenchymal stromal cells (MSCs) expressing collagen type II [14], which forms an anlage for individual bones in the endochondral skeleton [15]. MSCs undergo chondrogenic differentiation into chondrocytes while the mesenchyme located on the periphery forms the perichondrium [16]. Chondrocytes in the center of the cartilaginous template undergo hypertrophy and

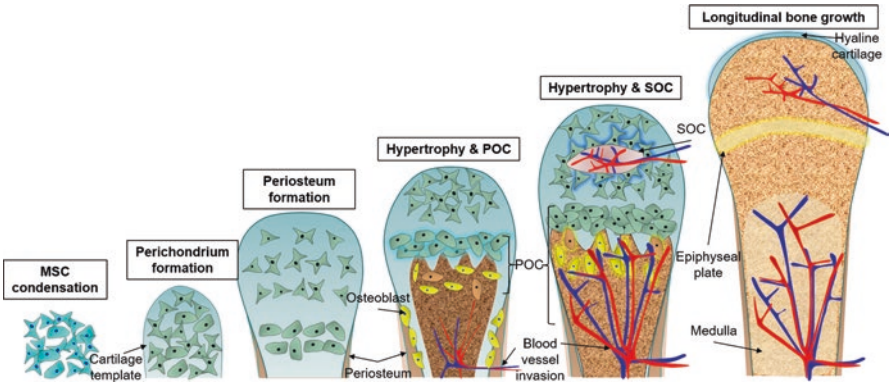

Fig. 1 Endochondral ossification (e.g., developing long bone). Schematic illustrating the phases of endochondral ossification, beginning with the condensation of MSCs and their chondrogenic differentiation to form an early cartilage template and perichondrium. Cells on the periphery undergo direct osteoblastic differentiation to form the perichondrium, while cells in the center proliferate rapidly and undergo hypertrophy, initiating mineralization of the cartilaginous matrix, which is then invaded by blood vessels, forming the primary ossification center (POC). The epiphyses are then invaded by blood vessels, forming a secondary ossification center (SOC), while the periphery maintains a stable cartilage phenotype, resulting in the formation of hyaline cartilage

begin to produce collagen type X, while cells in the periphery undergo direct osteoblastic differentiation to form an encircling bone collar [17, 18]. Hypertrophic cells then initiate bone synthesis by mineralizing the transient cartilaginous template, and the hypertrophic zone is invaded by blood vessels and an influx of cells, forming the primary ossification center [19]. The mineralized cartilage template is remodeled by osteoclasts, while osteoprogenitors differentiate into osteoblasts and lay down the osteoid of new bone. The developing epiphyses are then invaded by blood vessels, forming a secondary ossification center, while the periphery maintains a stable cartilage phenotype, resulting in hyaline articular cartilage surfaces seen within joints. The growth plate persists between primary and secondary ossification centers, propagating longitudinally to allow long bone growth before ossifying in early adulthood [12, 20].

Intramembranous ossification (Fig. 2) involves cells originating from the neural crest [21], which begins with the condensation of MSCs to form an ossification center, where they undergo direct osteoblastic differentiation [21]. These cells produce and secrete osteoid, which subsequently becomes calcified. Some osteoblasts become entrapped in this calcified matrix to become osteocytes. Bony spicules radiate out from the primary ossification center, while the entire region becomes surrounded by a compact layer of MSCs to form the periosteum. Cells on the inner surface of the periosteum also undergo osteoblastic differentiation and repeat the process, so that many layers of bone are formed. While fracture healing predominantly recapitulates endochondral ossification, intramembranous ossification also occurs subperiosteally, in both distal and proximal ends of the fracture to generate a hard callus from the periphery of the fracture towards the center of the fracture gap [1, 22]. The bridging

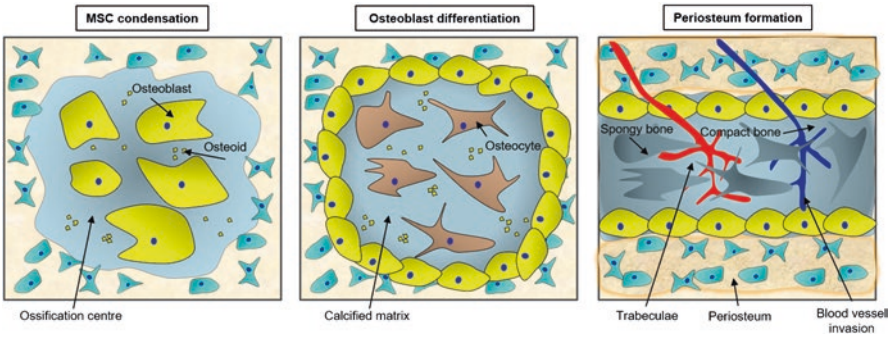

Fig. 2 Intramembranous ossification (e.g., developing calvaria). Schematic illustrating the phases of intramembranous ossification, which begins with the condensation of MSCs and the formation of ossification centers, where osteoblasts become entrapped in newly formed calcified matrix and become osteocytes. Subsequently, blood vessel invasion promotes surrounding osteoid to become calcified and the formation of trabeculae, while compact layers of MSCs on the surface of spongy bone become the periosteum, which in turn facilitates the formation of compact bone superficial to trabeculae

of this periosteal hard callus, which is a product of both endochondral and intramembranous ossification, ultimately provides fractures with a rigid structure to allow weight bearing—and is as such a hallmark of healing [23].

After fracture, long bones primarily heal following the route of endochondral ossification [1], which has led to an increase in the development of strategies aimed at recapitulating endochondral ossification in the field of tissue engineering using, for example, engineered cartilage as a template to promote bone formation [12, 24, 25].

Bone Healing: An Interplay Between Immunological and Mechanical Factors

Bone healing also recapitulates the process of bone remodeling. Remodeling is the process responsible for maintaining the general health and mechanical properties of bone tissue throughout the lifetime of an adult. Old or damaged bone is removed by bone resorbing osteoclasts while new bone matrix is produced by osteoblasts, allowing bone to withstand dynamic stress while repairing developing fatigue fractures [26]. In the healthy skeleton, a homeostatic balance between bone resorption and formation exists to maintain the function of bone throughout the lifetime of adults [11]. Homeostasis in bone remodeling is maintained by both immunological and mechanical factors.

Macrophages, an integral part of the innate immune system, have been shown to be a key facilitator of maintaining homeostasis in bone remodeling [27], not only by serving as the precursor to osteoclasts but also by coordinating osteoclast–osteoblast

coupling and by serving as a cellular canopy over bone remodeling sites [28, 29]. Key signaling molecules responsible for mediating osteoclast–osteoblast coupling include the receptor activator of nuclear factor κB (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG), which collectively form what is referred to as the RANK/RANKL/OPG axis [30]. Osteoblasts produce the transmembrane protein RANKL, which is responsible for inducing fusion of osteoclast progenitors into mature osteoclasts via binding to its receptor RANK on the surface of osteoclast progenitors [30]. OPG is a soluble decoy receptor, secreted by osteoblasts, responsible for maintaining a balance between bone resorption and formation. Adaptive immune cells, including B and T lymphocytes, can both positively and negatively influence bone homeostasis. For instance, T helper 17 (Th17) cells indirectly stimulate bone resorption through the production of interleukin 17 (IL-17), which stimulates RANKL expression on osteoblasts and stromal cells, and the synthesis of matrix-degrading enzymes [31]. Conversely, T helper 1 (Th1) and 2 (Th2) subsets of T lymphocytes have the capacity to inhibit osteoclastogenesis through their secretion of interferon gamma (IFN-γ) and interleukin 4 (IL-4), respectively [32]. Similarly, B lymphocytes can regulate bone homeostasis by producing OPG [30]. Taken together, it is clear that bone homeostasis is dependent on a complex interplay of factors produced by immune cells, which may, in part, be responsible for the increased risk of patients with chronic immune disorders, such as type 1 diabetes, to develop delayed healing and nonunion after fracture [33].

Mechanical factors are also important influencers of bone remodeling: for example, increased loading increases bone formation and decreases resorption, decreased loading decreases formation and increases resorption, while absolute immobilization stimulates resorption and halts formation [34]. The importance of mechanical regulation of bone remodeling is highlighted by Wolff's law, which states that the structure of bone will adapt to its mechanical usage [35] and can be aptly demonstrated in astronauts who lose bone mass after spending time in weightless environments due to reduced loading [36] and in tennis players who gain bone mass in their playing arm due to increased loading compared to their non-playing arm [37]. Given the importance of such immunological and mechanical factors in bone homeostasis, it is perhaps not surprising that these factors also play important roles during fracture healing. There are two main types of bone healing, namely primary and secondary fracture healing, which are dependent on the distance between the fractured bone ends, in addition to the mechanical stabilization of the fracture environment [38].

Primary Fracture Healing

Primary fracture healing, which seldom occurs, is characterized by minimal fracture gap and inter-fragmentary movement and can ensue either via contact healing or gap healing. Contact healing resembles bone remodeling, whereby macrophages play a key role in establishing osteoclast–osteoblast coupling to allow for resorption

and subsequent ossification. In this process, osteoclasts generate longitudinal cavities perpendicular to the long axis, which are later filled by osteoblasts, resulting in bone formation in the correct axial direction [39]. However, contact healing typically occurs only if the displacement between bone ends is less than 0.01 mm and the interfragmentary strain is less than 2% [1, 40]. Gap healing takes place when similar inter-fragmentary stabilization is achieved; however, the fracture gap is larger than in contact healing, but typically less than 1 mm [1]. In this process, the gap is first filled with an intermediate of lamellar bone oriented perpendicular to the long axis, which is later remodeled by a process resembling contact healing [41].

Secondary Fracture Healing

Secondary fracture healing, which is more clinically relevant and applicable to large defects, follows well-defined, histologically and mechanically distinct phases, namely hematoma formation associated with an initial proinflammatory phase, followed by the formation of granulation tissue, callus formation and remodeling, which ultimately results in bone formation via endochondral and intramembranous ossification [40, 42, 43] (Fig. 3). It has been suggested that the goal of secondary

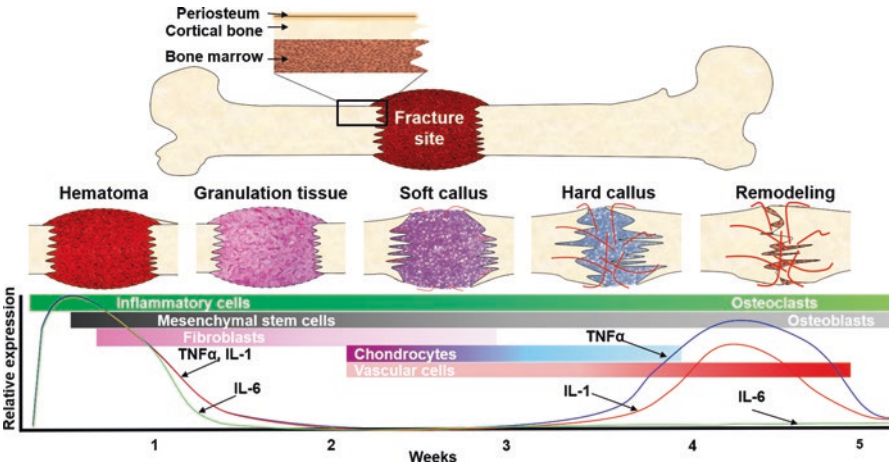

Fig. 3 Immunomodulation of fracture healing. Schematic illustrating the transient phases of fracture healing, which are progressively transformed from a proinflammatory hematoma to remodeled bone via a fibrocartilaginous intermediate, in part recapitulating both endochondral and intramembranous ossification. Temporal immunomodulation facilitates the smooth transition between phases of the healing cascade, orchestrating the influx of key cell types highlighted here with the relative expression pattern of some proinflammatory cytokines (TNFα, IL-1 and IL-6). Duality in cytokine functionality is also depicted with TNFα and IL-1, which are proinflammatory mediators initially, but later promote bone remodeling in the latter phase of bone healing. Remodeling can take significantly longer than shown here, particularly with larger injuries [44, 45]

fracture healing is to replace soft transient templates of bone tissue with more stable and rigid structures that allow weight bearing [41, 42].

The disruption of bone vasculature after fracture leads to the formation of a hematoma between bone fragments through activation of a plasma coagulation cascade and exposure of platelets to the extravascular environment, marking the beginning of a transient proinflammatory phase [40]. Rising importance has been given to the inflammatory phase of fracture healing as we shed more light on how the hematoma serves as the site where inflammatory cells can dock and control the expression of a temporally regulated cytokine pattern, which directs cell recruitment for subsequent stages of bone healing. As such, removal of the hematoma from fractures dramatically impacts on fracture healing, resulting in delayed healing. For example, in an ovine open tibial fracture model where the hematoma is removed in the first week post-injury, the quality of bone formation formed after 2 weeks is significantly reduced in comparison to undisturbed controls [46].

The fibrin-rich hematoma formed after fracture serves as the first transient matrix and docking site for the influx of inflammatory cells, mesenchymal cells, and endothelial cells which are attracted by resident macrophages, platelet-derived factors, complement fragments, and danger signals released from necrotic cells [26]. Among the inflammatory cells are neutrophils, which are the first responders to the fracture site [47]. While the complete role of neutrophils in bone healing has not been fully elucidated, it has been shown that neutrophils are responsible for recruiting a second wave of inflammatory cells, namely macrophages and T lymphocytes, through the secretion of proinflammatory and chemotactic mediators including IL-6 and monocyte chemoattractant protein-1 (MCP-1) [48]. Taken together, this group of inflammatory cells are responsible for initiating the subsequent stages in bone healing through the temporal regulation of cytokine patterns, which in many cases have bimodal functionality [49]. For example, TNFα (tumor necrosis factor α) is a potent proinflammatory cytokine, which is first produced by recruited inflammatory cells and resident macrophages. TNFα is now well regarded as a primary mediator of the proinflammatory phase within the hematoma, with its concentration peaking shortly after fracture (1–3 days) to promote MSC infiltration and proliferation [45, 50]. However, thereafter the concentration of TNFα drops for subsequent stages in bone healing until the remodeling phase where the level of TNFα is elevated again to facilitate osteoclast differentiation [45]. Consequently, cases where TNFα expression patterns are disturbed, particularly in the inflammatory phase, are those which are typically associated with delayed bone healing or nonunion [51]. Duality in cytokine functionality is not just specific to TNFα, for instance interleukin-1β (IL-1β) has a very similar bimodal expression pattern to TNFα [52]. Other examples of cytokine duality include IL-17, which is produced by Th17 cells and has both catabolic effects, by enhancing osteoclast-mediated bone resorption, and anabolic effects, by enhancing osteoblast-mediated bone formation [53]. While the initial proinflammatory fracture hematoma is critical for establishing the correct cytokine pattern to facilitate subsequent phases of bone healing, the effective "switching off" of the proinflammatory phase via anti-inflammatory mediators, such as IL-1 receptor antagonist (IL-1Ra) and IL-10, appears to be equally important to facilitate healing.

When the acute inflammation is cleared, a transient granulation tissue (7–14 days post-fracture) develops, whereby cells within the fracture hematoma gradually change the extracellular matrix into a proteoglycan and collagen-rich intermediate, while capillaries grow into the fracture site from endosteal circulation [54]. Facilitating angiogenesis is crucial during the formation of granulation tissue and later phases of bone healing. For instance, rats with femoral fractures treated with angiogenesis inhibitors fail to develop granulation tissue and exhibit minimal bone formation compared to control animals, which follow the typical healing process [55]. During the granulation tissue phase, recruited MSCs and fibroblasts are actively proliferating to prepare for the subsequent stages of healing where they will need to differentiate.

Soft callus formation is marked by chondrogenic differentiation of MSCs at the fracture site (2–3 weeks post-fracture). Chondrogenic differentiation is promoted by a combination of mechanical signals derived from micromotion provided by relative stability fixation techniques [56], the hypoxic microenvironment due to disrupted vasculature [57, 58], and macrophage-derived signals. Chondrogenic differentiation is induced and maintained by the coordinated expression of growth factors including transforming growth factor-β2 and -β3 (TGF-β2 and -β3), platelet-derived growth factor (PDGF), fibroblast growth factor-1 (FGF-1), and insulin-like growth factor (IGF) [57, 58]. Chondrocytes form a cartilaginous matrix rich in collagen type II and collagen type X, which serves as a scaffold for endochondral bone formation. As the soft callus develops with the help of fibroblasts to help pull the wound together and give it structure, intramembranous bone formation begins to take place in local areas that have improved blood supply, namely subperiosteally where periosteal stem cells differentiate directly into osteoblasts and form woven bone in both the distal and proximal ends of the fracture while advancing towards the fracture gap [22, 53]. The advancing bone front ultimately surrounds the external surface of the cartilaginous matrix, providing some degree of mechanical stability to the soft callus [59]. Initially, the soft callus matrix remains largely avascular to promote enough cartilaginous template for endochondral ossification [60]; however, as healing proceeds, the callus is invaded by endothelial cells, which promote vascularization into the fracture site [61]. Vascularization, stimulated by pro-angiogenic factors including vascular endothelial growth factor (VEGF), bone morphogenic proteins (BMPs), FGF-1, and TGF-β [62], promotes hypertrophy and the mineralization of the cartilaginous matrix, marking the end of the soft callus phase and beginning of the hard callus phase [63].

Hard callus formation recapitulates the events that occur in the secondary ossification center during long bone development whereby chondrocytes undergo hypertrophy and begin to calcify the cartilaginous matrix [64]. Concomitant with revascularization of the fracture site, osteoprogenitor cells, stimulated by osteogenic factors including BMPs secreted by MSCs [65], differentiate into osteoblasts, which facilitate the transition of the cartilaginous scaffold into a transient woven bone matrix. The exact source of osteoprogenitor cells remains ambiguous. Periosteal stem cells have recently been identified as the cell niche responsible for mediating intramembranous ossification subperiosteally [53], while bone marrow MSCs have

been known to contribute only to a limited amount of direct osteoblastic differentiation [66]. The hypothesis that osteoprogenitors originate from multiple sources including vasculature and surrounding local tissue stem cell niches [67, 68] is supported since a hard callus can also form, albeit to a limited extent, in the absence of MSCs and periosteum. The recent discovery of the periosteal stem cell [53] suggests that bone contains multiple resident stem cell niches, each with individual specialized functions.

In the final phase of bone healing, the irregular woven bone in the hard callus is remodeled into cortical and trabecular bone in a process that can take several months or even years to complete. Osteoclasts adhere to mineralized surfaces and, using a combination of proteinases and acid, are capable of degrading organic components such as collagen and demineralizing the matrix [69]. Bone resorption creates pits known as Howship's lacunae, which can be identified histologically, where osteoblasts, guided by macrophages, are able to deposit new bone [63]. Together with the aforementioned production of RANKL [70, 71], osteoblasts may also regulate osteoclast function via the production of macrophage colony-stimulating factor (M-CSF), which stimulates the differentiation of hematopoietic stem cells into osteoclast precursors [72]. Ultimately, remodeling can restore the original structure and function of the bone, completing the process of fracture healing.

Current Barriers to Successful Bone Healing

Given the multifactorial pathophysiology of fractures, a multitude of risk factors make it more likely that delayed healing and nonunions might develop. The United States Food and Drug Administration (FDA) defines a nonunion as a fracture that has not healed within 9 months of injury and shows poor progression of healing radiographically between months 6 and 9 [73]. However, the variable pathophysiology of fractures has also made it difficult to select the criteria that define a nonunion clinically, with citations ranging between 2 and 12 months [74]. Risk factors, including patient-related, fracture-related, and trauma-related, pose barriers to successful bone healing, which need to be overcome using innovative therapies that complement the intrinsic healing capacity of bone.

Patient-Related Risk Factors

Three of the most prevalent patient-related risk factors for impaired bone healing are diabetes mellitus, nonsteroidal anti-inflammatory drugs (NSAIDs), and smoking. Other patient-related risk factors including vitamin D deficiency [75], thyroid imbalance [76], hyperparathyroidism [77], and increasing age [78] are not covered in this review.

Diabetes mellitus was classically thought as a metabolic disease with high blood glucose levels [79], resulting from deficits in insulin production (type 1) caused by the autoimmune-mediated destruction of insulin-producing β-cells in the pancreas [80] or by a resistance to insulin (type 2) [81]. More recently, however, type 1 diabetes is increasingly being considered as an inflammatory disease characterized by dysregulated inflammation [82]. During type 1 diabetes, proinflammatory cytokines including IL-1β, IL-6, IL-18, and TNFα are significantly upregulated [83], and this inflammatory state appears resilient towards attempts to downregulate this inflammation once it has been induced [84]. Therefore, it is perhaps no surprise that the bone healing process, which is heavily influenced by proinflammatory mediators, is perturbed in patients with diabetes. Specifically, enhanced inflammation, and the inability to successfully resolve it due to diabetes, increases osteoclastogenesis during fracture healing, significantly increasing the likelihood of nonunion or delayed healing [84, 85].

NSAIDs, which inhibit the enzymes cyclo-oxygenase 1 and 2 (COX)-1/2 to varying extents depending on their chemical structure, are widely used drugs typically used to treat pain after surgery, including after fracture repair. However, usage of NSAIDs, including readily available drugs such as ibuprofen and aspirin, has been shown to be associated with an increased likelihood of developing fracture healing complications [86]. Prostaglandin E2 (PGE-2) is the most abundant prostaglandin in bone and plays a role via binding to its receptor, E prostanoid receptor 2 (EP2R), in the stimulation of bone formation, and in bone resorption via binding to EP4R [87]. NSAIDs can also lead to PGE-2 inhibition [88], impairing endochondral ossification, specifically limiting hypertrophy and bone deposition in both in vitro and ex vivo models [89]. However, the current clinical evidence is not sufficient to warrant discontinuation of all NSAIDs in all contexts of bone fracture and rehabilitation protocols but will certainly benefit from a greater number of randomized trials. For example, the association between nonunion after long bone fracture and duration of NSAID usage was recently assessed in several studies, but only one of these used a randomized controlled trial design [90–92]. Having used NSAIDs for 90 days postoperatively, the findings from the clinical studies suggest that NSAIDs have a detrimental effect on fracture healing. While the effects of NSAIDs are beneficial for pain management, it seems that their detrimental effect on fracture healing might be dependent on their relative use [86, 93]. Thus, further prospective randomized studies are required to fully elucidate the effects of short-term and long-term NSAID use, as well as cumulative doses, on fracture healing, and perhaps to find a balance between benefitting from the pain management aspect of NSAIDs without significantly impairing fracture healing.

A further important patient-related risk factor is smoking status. A recent meta-analysis, which sampled 40 studies incorporating over 8000 adults identified that smokers take 27.7 days longer (14.2–21.3) for union to occur after fracture and that smokers have greater than double (1.9–2.6) the risk of developing nonunion compared to non-smokers [94]. Nicotine and carbon monoxide are two constituents that particularly affect fracture healing. Nicotine decreases blood flow to the extremities due to

increased peripheral vasoconstriction [95], reduces microvascular perfusion [96], and increases blood viscosity and fibrinogen levels, which in turn increases the potential risk of microvascular clotting [97]. Additionally, nicotine directly damages osteoblasts and macrophages [98]. Carbon monoxide, with its 200-fold greater affinity for hemoglobin binding than oxygen, greatly reduces oxygen tension in tissues [99], exacerbating the nicotine-induced inhibitory effects on perfusion. Taken together, it is perhaps no surprise why smoking is such a significant risk factor for bone-healing complications. To minimize this risk, smoking cessation perioperatively is highly recommended [100]. The data here is categorically undebatable, with bone healing rates increasing in patients who give up smoking, particularly those who give up smoking for longer than 6 months postoperatively [101]. However, while these benefits are dependent on the length of smoking cessation, they also likely depend on lifetime smoking duration of the patient.

Fracture-Related Risk Factors

Fracture-related factors are dependent on the characteristics of the injury, including the location of the fracture, the extent of bone loss, the pattern of bone injury, and the condition of the soft tissue envelope surrounding the fracture. Several anatomical positions have been reported to have increased risk of nonunion. For example, comminution and poor interfragmentary cortical apposition in clavicle fractures have been associated with increased risk of nonunion [102]. Some locations within a single bone might also have a higher risk of nonunion. The poor blood supply associated with the distal tibia, the metadiaphyseal region of the fifth metatarsal, the tarsal navicular body and the scaphoid waist put these regions at higher risk of nonunion compared to other parts within the same bone [103–105]. Even though the exact quantity of bone loss required to develop nonunion has not been defined, the concept of a critical sized defect is often used, and thus, the extent of bone loss is also a significant risk factor for the development of nonunion. While these risk factors for the development of nonunion are inherently inevitable, there are other fracture-related risk factors that arise from fracture management and can thus be addressed.

The risk of nonunion might also be elevated through inadequate fracture management despite the high standards set by modern fracture stabilization techniques. When fixation strategies are used, excessive stripping of the periosteum might compromise native periosteal stem cell niches and dampen the fracture-healing capacity. Fractures that are not stabilized appropriately might also develop atrophic or hypertrophic nonunion. Inappropriately rigid stabilization with insufficient interfragmentary movement might inhibit bone growth leading to atrophic nonunion, while too much micromotion and interfragmentary strain can lead to large amounts of connective tissue being formed, resulting in a hypertrophic nonunion [106].

Trauma-Related Risk Factors

Concomitant with the severity of the fracture is the extent of the trauma-induced damage to the surrounding soft tissue. Maintaining the health of the surrounding tissue envelope aims to preserve the blood supply for fracture healing while extensive damage might limit revascularization during the bone healing process. Another trauma-related risk factor for nonunion is infection, presenting one of the biggest clinical challenges of the twenty-first century modern trauma medicine.

Osteomyelitis is an infectious disease that triggers inflammation, caused primarily by *Staphylococcus aureus* and *Staphylococcus epidermidis*, which often leads to bone destruction and bone loss [107, 108]. Infection is predominantly caused via open fractures, where there is a breach of the skin during the injury itself, permitting microorganisms to enter the wound and to colonize the bone tissue. A much reduced, although not insignificant, risk of infection occurs during surgical procedures themselves, for example, with prosthetic joint replacements or implantation of fracture fixation devices, where the surface of implants themselves are at potential risk of bacterial colonization.

In the general population, the incidence of bone infection after fracture can vary between 1.8 and 27% depending on the fracture type (closed vs. open) and location; however, with lower extremity open fractures, e.g., the tibia, demonstrating the highest incidence and being most affected [109–111]. Osteomyelitis also has an incidence rate of up to 2.4% in total hip arthroplasties and up to 3% for total knee arthroplasties [112–114]. In subpopulations with predispositions to infection, including patients with underlying disease such as diabetes or peripheral vascular disease, the incidence of osteomyelitis can be significantly greater [115]. The source of infection can be either contiguous, where osteomyelitis originates from trauma, direct inoculation during surgery, and surrounding infectious tissues, or hematogenous, where osteomyelitis arises from existing infection in another part of the body and is facilitated access to the fracture site via the circulating blood. In adults, 80% of osteomyelitis cases are contiguous, while in children the source is predominantly hematogenous [116, 117].

The pathogenesis of osteomyelitis follows targeting of bone healing processes and is mediated, in part, by microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and, in part, by the toxins they produce. Infection begins with colonization, the attachment of *Staphylococcus* to the surface bone or the surface of implants, once coated with host plasma proteins. This ‘race for the surface’ is mediated by the presence of MSCRAMMs, such as protein A (SpA) or fibronectin and collagen binding protein (FnBP A/B) which interact with bone cells, the extracellular matrix (ECM) and plasma proteins. Attachment of staphylococci to the surface of bone or implant facilitates biofilm formation, which are colonies of microorganisms enveloped in ECM that allow the infection to persist during treatment [118]. MSCRAMMs may also be secreted; for example, SpA can bind directly to osteoblasts, mediate cell death, and inhibit bone formation [119–121]. FnBPs can mediate internalization via the osteoblast integrin receptor α5β1 (the fibronectin

receptor) [122, 123], which can lead to apoptosis of the cell via binding to TNF-related apoptosis-inducing ligand (TRAIL), and activation of IL-6, IL-12, and CSF, which further exacerbate bone loss by enhancing inflammation (or impairing healing) [124, 125]. *Staphylococcus* can also persist intracellularly to evade the immune system [126] and even reside internally within hematopoietic cells, hijacking osteoclastogenesis to further the effects of bone resorption [127, 128]. During osteomyelitis, many toxins are also produced which negatively impact the bone healing process. For instance, *S. aureus* produces toxic shock syndrome toxin 1 (TSST-1), coagulase, Panton-Valentine leucocidin (PVL), hemolysins (Hla), and phenol-soluble modulins (PSMs) [129]. Through an unknown mechanism, TSST-1 mediates immune evasion and is also a mediator of osteoclast activation while not being directly cytotoxic towards them, resulting in increased bone resorption [130]. Hla, which lyses red blood cells, typically serves as an antigen for the innate immune system to detect; however, in osteomyelitis it is downregulated, contributing to the quiescence of bone infection, allowing the infection to evade the immune system [127]. The production of coagulase, which converts fibrinogen to fibrin, provides *S. aureus* with a physical shield against the innate immune system [131]. In mouse models, PVL has been shown to be responsible for the spreading of osteomyelitis [132], while PSMs contribute to the severity of infection. Taken together, these mechanisms allow *Staphylococcus*-induced osteomyelitis to prolong infection and evade the immune system while the natural processes of bone healing are hijacked, leading to bone destruction and bone loss.

Conclusion

Bone attempts to self-heal in response to injury by recapitulating the biology of bone development and bone homeostasis. Specifically, an acute proinflammatory hematoma is established for the docking of immunomodulatory cells, which set up highly regulated transient cytokine patterns to facilitate the transformation of the fracture hematoma to remodeled bone via a fibrocartilaginous intermediate. Despite its intrinsic healing capacity and modern orthopedic fixation methods to provide mechanical stability, large bone defects do not always heal successfully, which might result in delayed healing and nonunion. Barriers to successful healing, which arise from patient-, fracture-, and trauma-related risk factors, can be minimized to increase the likelihood of healing. However, bone infection is still a major clinical burden, exasperating patients with fractures due to its capacity to hijack and impact the bone healing process, and an alarming clinical concern due to the emerging prevalence of antibiotic resistance. Concomitant with our increasing understanding of the immunomodulation of bone healing, the development of novel biomaterials to serve in place of bone autografts may also permit the local delivery of immunomodulators and/or antibiotics, thus paving the way for innovative therapeutic strategies aimed at restoring a pro-healing environment in patient populations at increased risk of healing complications.

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