Fracture healing under healthy and inflammatory conditions

Lutz Claes, Stefan Recknagel and Anita Ignatius

Abstract | Optimal fracture treatment requires knowledge of the complex physiological process of bone healing. The course of bone healing is mainly influenced by fracture fixation stability (biomechanics) and the blood supply to the healing site (revascularization after trauma). The repair process proceeds via a characteristic sequence of events, described as the inflammatory, repair and remodeling phases. An inflammatory reaction involving immune cells and molecular factors is activated immediately in response to tissue damage and is thought to initiate the repair cascade. Immune cells also have a major role in the repair phase, exhibiting important crosstalk with bone cells. After bony bridging of the fragments, a slow remodeling process eventually leads to the reconstitution of the original bone structure. Systemic inflammation, as observed in patients with rheumatoid arthritis, diabetes mellitus, multiple trauma or sepsis, can increase fracture healing time and the rate of complications, including non-unions. In addition, evidence suggests that insufficient biomechanical conditions within the fracture zone can influence early local inflammation and impair bone healing. In this Review, we discuss the main factors that influence fracture healing, with particular emphasis on the role of inflammation.

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Introduction

Fractures are one of the most frequent injuries of the musculoskeletal system. Although fracture treatment has improved considerably in recent decades, a large proportion of all fractures still display delayed healing and complications including non-union. The outcome of fracture-healing depends on a number of factors, such as trauma severity, the quality of fracture reduction (realignment), fracture fixation technique and presence of comorbid diseases. Improved fracture healing is achieved if the natural healing process is not compromised, and through creation of ideal biological and mechanical conditions for repair.[1] Thus, optimal repair requires conservative[2] or surgical[3] stabilization of the bone fragments using minimally invasive techniques. Even when taking these principles into consideration, often treatment does not lead to optimal healing, particularly when additional injuries such as severe soft tissue trauma[4] or polytrauma[5] accompany the fracture, or if the patient has a comorbid disease.[6] Nevertheless, if fracture treatment is optimized and no other serious impairments are present, bone can heal without scar formation and regain its original form.[7]

Knowledge of the complex physiological process of bone healing is a prerequisite for optimal fracture treatment. However, the large number of variables that affect bone healing in patients and the difficulty in defining an exact end point of fracture repair hampers clinical studies.[4,8] As a result, most of our present knowledge of fracture healing is based on animal studies. Although the healing capacity and speed is greater in small animals than in large animals and humans, the general mechanisms of repair seem to be similar. New animal models and methods of studying bone repair have become available, enabling a clearer insight into molecular and genetic aspects of fracture healing to be obtained.[9] Additionally, new biomechanical approaches enable better characterization of fracture fixation and healing outcome in these models.[10,11]

To comprehensively review all aspects of fracture healing would be difficult, owing to the complexity of this process. Furthermore specific facets of this process have been described elsewhere.[1,7,12–15] Thus, this Review focuses on the main factors affecting fracture healing, the most important of which include trauma severity, fracture stabilization, inflammatory processes and revascularization of traumatized tissue, and the interactions between them (Figure 1). The effects of these factors, particularly inflammation, on bone formation and mechanobiology will be discussed.

Biomechanics of fracture fixation

The aim of fracture fixation is to anatomically align the bone fragments and achieve sufficient stability to enable undisturbed fracture healing. The fixation technique used dictates the degree of interfragmentary movement that occurs under external loading and muscle activity, which in turn determines the mechanobiology of bone healing (Figure 1).[15,16] Low interfragmentary movement, with resulting low interfragmentary strain, induces intramembranous bone formation (the direct conversion of mesenchymal tissue into bone). Moderate interfragmentary movement leads to endochondral ossification (in which a cartilaginous matrix intermediate is converted to bone; also known as callus healing), whereas high

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Institute of Orthopedic Research and Biomechanics, Center of Musculoskeletal Research, University of Ulm, Helmholtzstraße 14, 89081 Ulm, Germany (**L. Claes**, **S. Recknagel**, **A. Ignatius**).

Correspondence to: L. Claes
lutz.claes@uni-ulm.de

Key points

- Fracture healing is a complex, highly regulated process with consecutive and closely linked phases of inflammation, repair and remodeling
- Optimal fracture healing requires suitable biological as well as biomechanical conditions
- The mechanical environment considerably influences tissue differentiation during bone healing: stable fracture fixation induces direct bone formation, moderate stability provokes endochondral ossification, whereas unstable fixation inhibits bone healing
- The immune system is intimately involved in the fracture healing process, especially during the early inflammatory healing phase
- Disorders associated with systemic inflammation, such as diabetes mellitus, trauma, sepsis and rheumatoid arthritis, can prolong or disturb fracture healing and increase the risk of non-unions by incompletely understood mechanisms

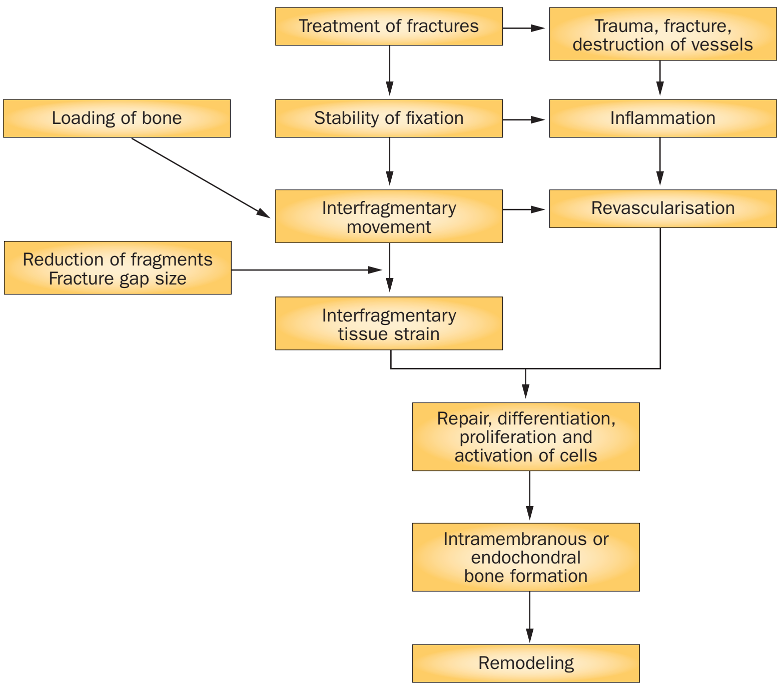

Figure 1 | Main factors affecting the fracture healing process. Trauma and fracture lead to blood vessel rupture inside bone and surrounding soft tissue, and activate cells, all of which contribute to initiation of the inflammatory cascade. Treatments, such as intramedullary nails, can initially increase damage. Revascularization starts at the periosteum and progresses towards the hematoma, providing the healing area with cells, cytokines and growth factors. The fracture fixation technique affects the interfragmentary movement that occurs upon loading of the bone. Interfragmentary movement causes interfragmentary tissue strain, which also depends on the reduction of the bone fragments, and has a direct effect on the mechano-sensitive cells as well as on inflammation and revascularization. Together, these effects drive the differentiation, proliferation and activation of cells and lead to intramembranous or endochondral bone formation and healing. Finally remodeling processes lead to reshaping of the fracture and a reconstruction of the bone.

interfragmentary movement inhibits bone healing.[15,16] Splinting and compression are the two principle forms of stable fracture fixation.[3,14,17]

External splinting

Most shaft fractures are stabilized with plaster casts or braces, which allow a large degree of interfragmentary movement[2,17] and, thus, induce healing with a callus. An external fixator is used instead of a plaster cast or brace if a fracture is accompanied by an open soft tissue wound or infection. The interfragmentary stability achieved by external fixator splinting depends on various parameters, including the distance between the bone and the fixator body, the diameter and spacing of the screws, and the number of screws used.[17,18] Stability ranges from flexible to rigid,[17–19] and usually external fixator splinting induces callus healing (Figure 2). External fixators that are too flexible can lead to delayed fracture healing.[20–23]

Internal splinting

Intramedullary nails

Intramedullary nails are internal splints that are placed into the medullary (marrow) cavity of long bones. Interlocking nails, which incorporate screws or bolts at each end of the splint to fix them securely in place, are normally used to prevent rotational instability. After reaming—enlargement of the intramedullary canal by drilling—the medullary cavity, the nails can be implanted in a press-fit manner,[24] or thinner nails can be used in an unreamed technique.[3] The unreamed technique preserves more of the intramedullary blood supply, but frequently results in unstable fracture fixation,[3,25–27] which increases the risk of delayed healing. Therefore, thicker nails, which can be implanted by a minimally invasive technique, are usually used at present.

Internal fixator plates

Internal fixators are plates that are normally rigidly fixed close to the bone surface using locked screws.[14] These systems are mainly used in epiphyseal and metaphyseal fractures because locked screws enable better fixation of the plate to trabecular (cancellous or spongy) bone, which is softer than the cortical bone that makes up the diaphysis, and particularly to osteoporotic bones. Internal fixators allow some interfragmentary movement in the area of the bone located opposite the fixator, stimulating callus healing, but suppress bone formation directly adjacent to the plate (Figure 3).[15] To prevent inhibition of bone formation, new systems have been developed that allow some axial interfragmentary movement, stimulating bone formation across the fracture line.[28]

Compression plates

Interfragmentary compression can be achieved with lag screws and compression plates (Figure 4a),[3] and, similar to internal fixator plates, this technique is most often used in the treatment of metaphyseal and epiphyseal fractures. Under compression of the fragments, direct bone healing by intramembranous trabecular bone formation occurs without external callus formation (Figure 4).[29]

Revascularization

In the first few days following fracture or osteotomy the total blood flow to the affected area of bone is markedly reduced (Figure 5),[30,31] owing to the rupture of blood vessels and physiological vasoconstriction in both the periosteal and the medullary vessels in response to trauma. However, during the fracture repair phase intraosseous and extraosseous arterial circulation increases.[32] The blood supply peaks above pre-injury levels at 2–4 weeks post-fracture in rats,[31–33] and decreases gradually thereafter (Figure 5c); although no quantitative studies have been performed in humans, the timescale is like be similar in patients with fracture. In contrast to the normal centrifugal blood flow from the medullary area in intact bones, after fracture and callus formation the blood supply mainly derives from surrounding soft tissue.[34–37]

Fracture fixation alters the blood flow at the fracture site because the blood supply to the fracture hematoma, the bone cortex and the soft tissue is affected by the operative procedure used.[38,39] Intramedullary nailing temporarily impedes the local blood flow,[38–40] independent of whether nail implantation was performed with or without reaming of the medullary channel.[31] Compression plates with a large bone contact area can disturb the periosteal and cortical blood supplies.[3,14] The least disruption of the blood supply is achieved by using casts, braces, or external or internal fixators.[39]

Revascularization of the fractured bone seems to be dependent on fracture fixation stability. During the early stages of fracture healing, greater interfragmentary movement can promote revascularisation,[41] whereas in the later phases of repair more stable fixation is associated with improved blood flow.[41,42]

Additional local or systemic trauma can decrease blood flow and impair fracture healing. Moderate soft tissue traumata has been demonstrated to reduce the blood supply for the first days after fracture in animal models,[32] without affecting the outcome of bone healing;[30] however, periosteal devascularisation,[43] or extensive muscle injury,[44] considerably reduced fracture healing in a rat model. Furthermore, in studies also performed in rats, combined fracture, thoracic and soft tissue trauma resulted in decreased healing compared with fracture alone.[45] Moreover, no fracture healing occurred after treatment with a compound that prevents angiogenesis (the methionine animopeptidase-2 inhibitor TNP-470).[46] Together, these data suggest that adequate revascularization of tissue at the fracture site is required for effective fracture repair.

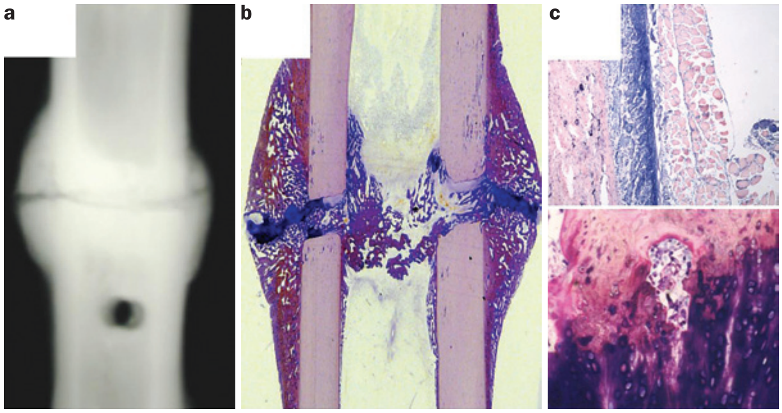

Figure 2 | Secondary diaphyseal bone healing in a sheep tibia osteotomy model. **a** | Radiograph demonstrating callus healing of the osteotomy after flexible fixation using an external fixator with moderate interfragmentary strain. **b** | Longitudinal histological section of the osteotomy site shortly before bony bridging (9 weeks post-operation), showing mainly calcified periosteal (peripheral; red) and little endosteal callus formation as well as fibrocartilage (purple) at the level of the osteotomy line (Paragon staining; magnification 3x). **c** | Longitudinal histological sections from the fracture healing zone (Paragon stained; magnification 20x) demonstrating early intramembranous bone formation adjacent to the periosteum (4 days post-operation; blue) where interfragmentary movement causes minimal tissue strain (top panel), and endochondral bone formation in the peripheral callus area at the borderline between fibrous cartilage (purple) and calcified new bone (light red) where interfragmentary strain is higher (bottom panel).

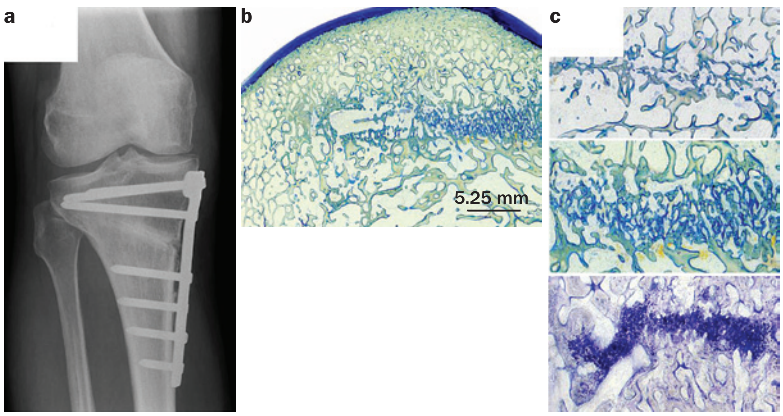

Figure 3 | Images demonstrating the metaphyseal bone healing process. **a** | Radiograph of a metaphyseal tibia osteotomy in a patient stabilized by a plate with interlocking screws, which allow better fixation of the plate to the soft trabecular bone. **b** | Longitudinal histological section from a metaphyseal fracture healing model (3 mm osteotomy gap) in the trochlea region of the distal sheep femoral condyle with low interfragmentary strain on the left and moderate interfragmentary strain on the right side (8 weeks post-operation; Paragon stained; magnification 2x). **c** | Histological sections showing contact healing and gap healing of trabecular bone (Paragon stained; magnification 6x). Contact healing results in a dense horizontal line of bone formed by new trabeculae that are thicker and more densely arranged (top panel), but which will later be remodeled. During metaphyseal gap healing thick trabeculae proceed (in a vertical direction) from both sides of the gap and unite without callus formation (middle panel). The tissue that develops in the fracture gap depends on the interfragmentary strain, and gap healing under large interfragmentary strain results in a tissue dominated by fibrocartilage (purple; bottom panel).

Phases of fracture healing

Fracture healing follows a characteristic course, which can be divided in to three partially overlapping phases: inflammation, repair and remodeling (Figure 5a). This sequence of events has been observed in many animal species, being best described in rats. Therefore, the following discussion focuses on the rat fracture healing model. The fracture healing process is similar in larger animals and humans, but occurs over a longer time-course.

Inflammatory phase

Fracture leads to blood vessel rupture inside bone and in the surrounding soft tissue, as well as damage to other cells and tissues, which promotes the initiation of the inflammatory cascade and fracture healing.[47] Subsequently, the soft tissue surrounding the fracture takes on usual characteristics of acute inflammation, with vasodilatation and exudation of plasma and leukocytes.[7,48] The ends of the broken bones die off to a variable distance from the fracture depending on the degree of trauma,[7] and within the fracture gap fibrinogen is converted into fibrin, leading to fracture hematoma formation.[49] This hematoma is characterized by hypoxia and low pH, and

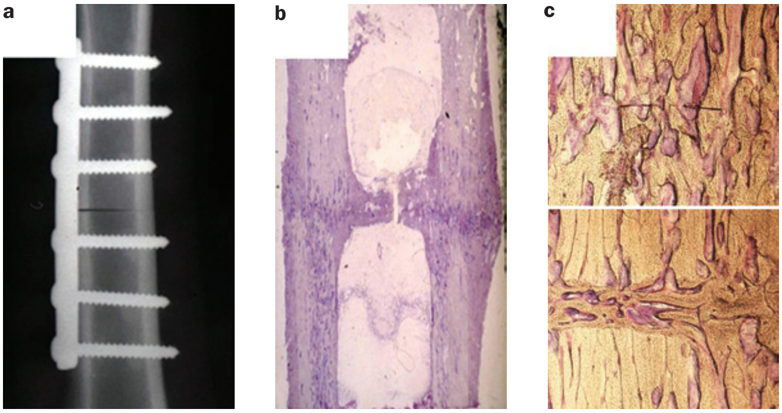

Figure 4 | Primary diaphyseal bone healing in a sheep metatarsal osteotomy model. **a** | Radiograph of the osteotomy after rigid fixation using a compression plate, which provides the necessary stability for primary, direct bone healing. **b** | Longitudinal histological slice 24 weeks after surgery (Paragon stained; magnification 4x). The osteotomy site demonstrates differences in repair in locations with a gap (adjacent to the plate; left-hand side) or contact (opposite the plate; right-hand side) between the fragments. **c** | Histological section demonstrating contact healing with osteons crossing the osteotomy line (top) and gap healing with woven bone filling the gap and osteons beginning to bridge the osteotomy (bottom, Paragon staining; magnification 20x). Permission obtained from Lippincott Williams & Wilkins © Claes, L. E. & Ito, K. in *Basic Orthopedic Biomechanics and Mechano-Biology*, 3rd edn (eds Mow, V. C. & Huiskes, R) 563–584 (Lippincott Williams & Wilkins, Philadelphia, 2005).

houses peripheral blood-derived inflammatory cells,[50] together with proinflammatory and anti-inflammatory cytokines (Figure 6).[47]

The hematoma acts as a temporary scaffold for the active invasion of additional inflammatory cells. The first cells recruited are polymorphonuclear neutrophils (PMNs), which are attracted by dead cells and debris[51] and rapidly accumulate during the first hours after injury. PMNs are short-lived (around 1 day), but secrete several chemokines (such as C-C motif chemokine 2 [CCL2] and IL-6) that attract longer-lived macrophages.[52–54] PMNs are thought to have a negative effect on the fracture healing process,[52,55] whereas macrophages seem to have a positive influence on this process. The resident macrophage cell population (osteomacs)—present on the endosteal and periosteal surfaces in close proximity to bone lining cells of healthy unfractured bone—seem to be pivotal for intramembranous bone formation during fracture healing (Figure 6).[56] By contrast, inflammatory macrophages recruited to the site of injury have a particular influence on endochondral ossification.[54] After a period of macrophage recruitment and activity, lymphocytes migrate into the fracture callus and initiate the adaptive immune response.[53]

A large number of proinflammatory cytokines (IL-1, IL-6, TNF, receptor activator of nuclear factor κB ligand [RANKL], macrophage colony-stimulating factor 1) and members of the transforming growth factor (TGF)-β superfamily (bone morphogenetic protein [BMP]-2, BMP-4, BMP-5, BMP-6) are released early in the inflammatory phase.[13,57,58] In addition, angiogenic factors (angiopoetin-1, and, later, vascular endothelial growth factor) are released, as a result of the hypoxic conditions created by the disturbed vascularisation.[57] As described above, revascularization is essential for fracture healing, and angiogenesis is required to re-establish normoxic conditions, remove debris and supply the fracture zone with cells and mediators.[59] Endothelial cells migrate from pre-existing periosteal vessels, towards the bone ends, and into the hematoma to form new blood vessels.[60] Blood vessels also provide access to an excellent source of osteoprogenitor cells, which are thought to derive from pericytes.[61] Subsequently, fibroblasts appear at the fracture site and are responsible for new collagen production and crosslinking in the hematoma. The hematoma is gradually replaced by a granulation tissue rich in collagen fibers, cells and invading capillaries.[7]

The acute inflammatory response occurs over the first 7 days after fracture in rats (Figure 5a), and maximum levels of IL-6 and IL-1β are reached within the first 24 h.[62–64] This early inflammatory reaction, with its complex network of interactions between molecular factors, immune cells, resident tissue cells and progenitor cells, is thought to initiate the repair cascade by stimulating angiogenesis, attracting and promoting differentiation of mesenchymal stem cells (MSCs), and enhancing extracellular matrix synthesis.[13,54,58] Evidence suggests that MSCs might have both local and systemic anti-inflammatory effects during fracture healing,[65] indicating that a negative feedback loop might control the inflammatory response. Nevertheless, a certain degree of inflammation seems to be required, as a marked impairment in fracture healing has been observed after treatment with anti-inflammatory drugs such as cyclooxygenase-2 (COX-2) inhibitors.[66] COX-2 is expressed rapidly after fracture,[67] and is the key rate-limiting enzyme in the conversion of arachidonic acid into various prostaglandins, which are known to be strong inducers of inflammation.[68] Furthermore, COX-2 activity has been shown to promote angiogenesis and differentiation of MSCs into osteoblasts during fracture healing.[66]

Repair

The nature of the repair phase is dependent on mechanical conditions in the fracture healing zone (primary or secondary bone healing) and the anatomical location of the fracture (metaphyseal–epiphyseal trabecular bone healing or diaphyseal callus healing).

Direct, primary bone healing

Primary cortical bone healing occurs only under extremely low interfragmentary movement or if the bony fragments are under compression.[14,69] Most often compression plates and lag screws create the necessary stability for primary cortical bone healing.[3] If such stability is achieved, fracture surfaces in contact and under compression are bridged by Haversian systems (or osteons; Figure 4c), similar to the normal bone remodeling process.[7,14,69,70] Osteoclasts resorb bone, creating tunnels from one side of the fracture to the other, which enables the in-growth of blood vessels. Subsequently, precursor cells are recruited and differentiate into bone-forming osteoblasts,[14,63] which create new osteons connecting both fragments (Figure 4b–c). Where a gap exists between fracture surfaces, woven bone is laid down between the fragments and vascularized from the periosteum and medulla, before the fracture is bridged by osteon formation (Figure 4c).[7,14] Bone healing by Haversian systems is slow, and considerable time is taken until the healing zone gains sufficient strength to allow removal of load-bearing implants. As primary bone healing is not associated with a major influx of inflammatory cells, it might be less affected by systemic inflammation.[52]

Indirect, secondary bone healing

Stabilization of diaphyseal fractures using plaster of Paris, braces, or operative treatment with intramedullary nails, external fixators or bridging plates allows considerable interfragmentary movement upon loading of the broken bone.[2,17,19,71,72] This low degree of stability (relative to that achievable by compression) stimulates primary development of a predominantly soft callus, which is secondarily transformed into a bony callus (Figure 2).[7,12,30] Thus, periosteal callus formation is the dominant type of bone formation. Callus formation partially overlaps with the inflammatory phase (Figure 5). Intramembranous bone formation starts as early as 3–7 days after injury in rats at some distance from the avascular fracture ends at the periosteum (Figure 2c). The osteoblasts involved in intramembranous bone formation are believed to derive from periosteal precursor cells,[73] and periosteal stripping diminishes the capacity for callus formation.[43] Bone formation is assumed to start in a region where the periosteum and vascularization are not disturbed by the trauma, and where interfragmentary movement causes minimal tissue strain.[16]

Further callus growth is driven by chondrocytes, with cartilaginous tissue forming 7–10 days after fracture in rats. Such tissue formation progresses towards the fracture over time (Figure 2b; Figure 5). Within the fracture gap and between the cartilaginous callus wedges, connective and granulation tissue is formed. Soft callus size and cartilaginous tissue content increases with increasing interfragmentary movement,[7,23] and reaches maximal volume at approximately 14 days post-injury in rats (Figure 5d).[30]

Cartilaginous tissue formation could be the result of insufficient blood supply to newly developed tissue that lies a considerable distance from the undisturbed periosteum and higher tissue strain closer to the fracture line, which both diminish the potential for new vessel formation.[35,42] The resultant low oxygen tension impairs osteoblast activity but allows chondrocyte differentiation and proliferation.[7,74]

After approximately 10–14 days of proliferation in rat fracture healing studies, chondrocytes become hypertrophic, release calcium and undergo apoptosis,[12,63,75] similar to the mechanism that occurs in the growth plates during endochondral ossification.[12] Upon bridging of the fracture by the cartilaginous callus wedges, the interfragmentary movement and tissue strain during loading of the fracture is markedly reduced,[22] allowing blood vessel to invade the calcified cartilage and resulting in hypervascularization (Figure 5c).[32,35,42] These blood vessels enable the recruitment of MSCs and monocytes. Whereas monocytes differentiate into osteoclast-like cells, which resorb the calcified cartilage, MSCs differentiate into osteoblasts,

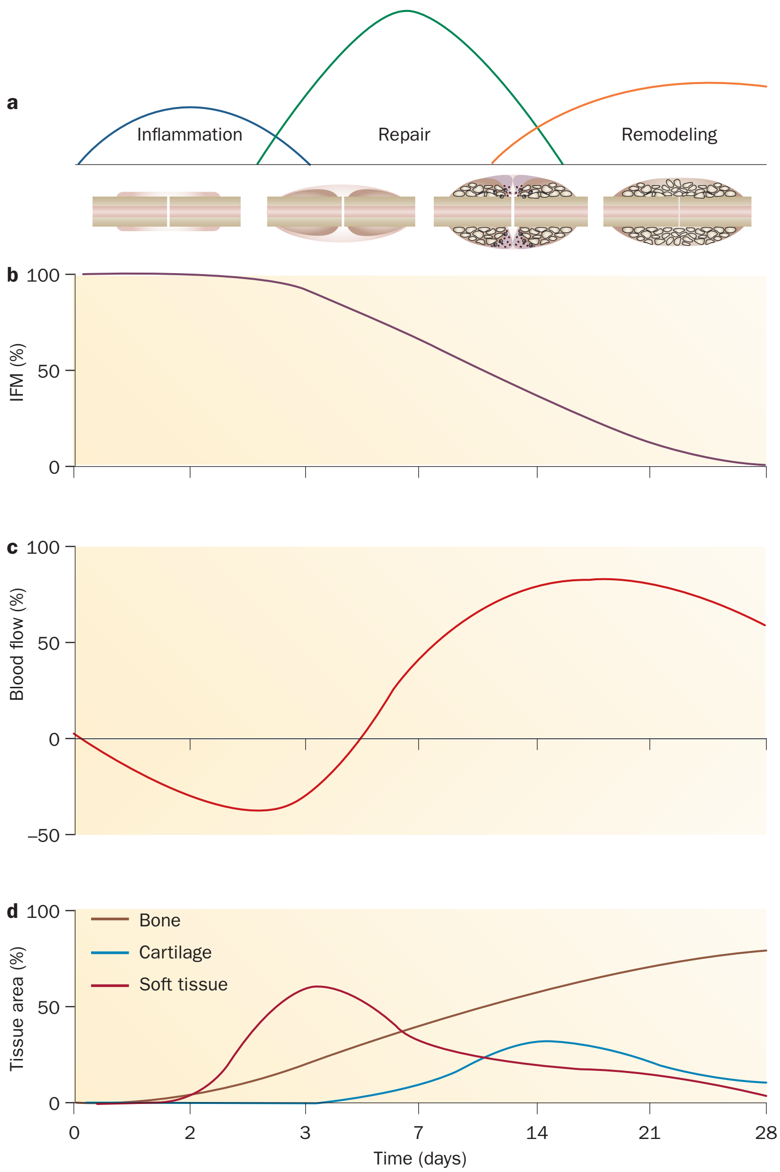

Figure 5 | Time course of fracture healing events in rats. **a** | Fracture healing can be divided into three overlapping phases: inflammation, repair and remodeling. The inflammatory cascade is initiated by cell and tissue damage, and persists for roughly 4 days. The repair phase begins with intramembranous bone at the periosteum some distance from the fracture, which drives callus formation. The callus grows and progresses in direction to the fracture. At larger distance from undamaged vessels hypoxic conditions allow only chondrocyte proliferation and endochodral ossification. Blood vessels invade the cartilaginous callus, osteoclast-like cells resorb the calcified cartilage and osteoblasts build new bone. After bony bridging of the fracture, callus diameter decreases and bone is remodeled. **b** | IFM varies over the course of fracture healing. The fracture is least stable immediately after fixation and IFM only decreases considerably when new bone is created during the repair phase. Repair and remodeling eventually restore bone structure and IFM ceases. **c** | Blood flow in the fracture zone is initially reduced as a result of vessel rupture and vasoconstriction. During the remodeling stage, the tissue becomes hypervascularized owing to new vessel formation, enabling recruitment of cells and nutrients, which is essential to repair. Blood flow is represented as percentage change from pre-fracture levels. **d** | Tissue composition varies throughout fracture repair. Initially, soft tissue predominates, but gives way to cartilage after around 7–14 days. The cartilage is then replaced by bone. Bone formation increases soon after fracture in regions least affected by the trauma and with low interfragmentary strain, and progresses as blood flow increases and IFM decreases. Abbreviations: IFM, interfragmentary movement.

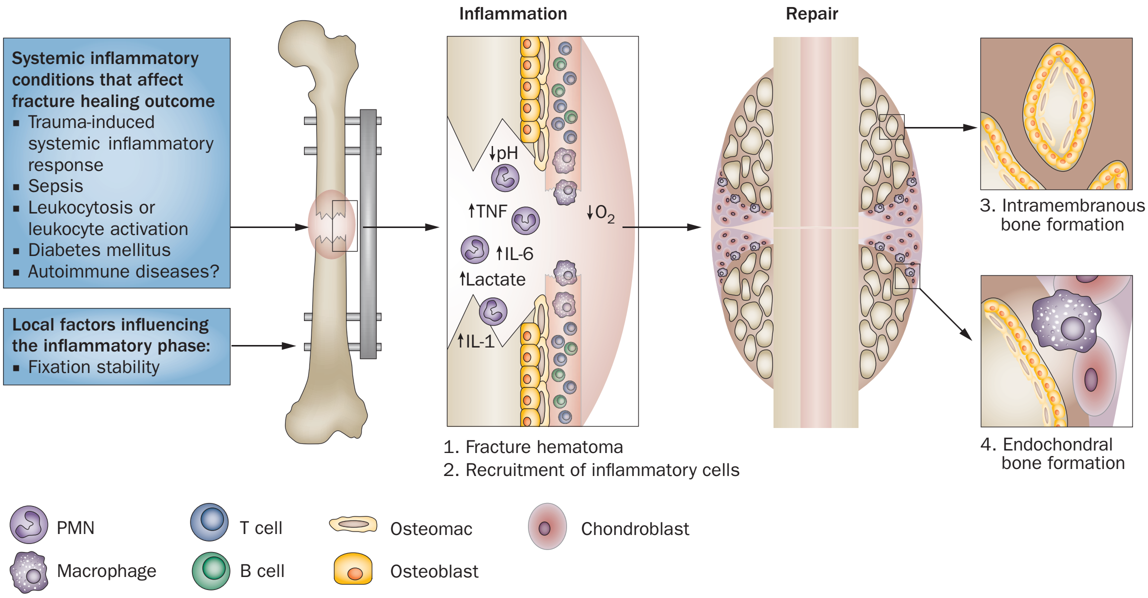

Figure 6 | Schematic representation of inflammation and repair during fracture healing. Immediately after the initial trauma, the fracture hematoma is formed as a result of blood clotting. The fracture hematoma is characterized by hypoxia and low pH, and contains proinflammatory and anti-inflammatory cytokines together with inflammatory cells from the peripheral blood (1). During the initial inflammatory phase of bone healing, immune cells are rapidly recruited to the site of injury (2), neutrophils being the first cells to invade the callus, followed by macrophages and lymphocytes. During the repair phase, osteomacs are pivotal for osteoblast-driven mineralization in zones of intramembranous ossification (3), whereas inflammatory macrophages mainly contribute to endochondral bone formation (4). Several systemic (severe trauma, leukocytosis, diabetes mellitus and possibly autoimmune diseases) and local factors (fixation stability) affect inflammatory processes at the fracture site, and can result in impaired fracture healing. Abbreviations: PMN, polymorphnuclear neutrophils.

which fill the resorption lacunae with new bone.[63] These processes lead to the formation of woven bone with a trabecular structure (Figure 2b). After bony bridging, the strain on tissue in the fracture gap and between the remaining callus wedges is sufficiently low to enable replacement of connective and granulation tissues through intramembranous bone formation.[15] Depending on fracture fixation stability, this process occurs in rats 28–35 days after injury.[30,63] Parallel to periosteal callus healing, bone formation also occurs in the medullary region. The amount of bone produced is less than in the periosteal region, and medullary bone formation seems relatively independent of mechanical influences.[7] A number of morphogenetic signals guide the repair process and dictate the temporal progression through the phases of callus formation;[13] the complex molecular mechanisms involved exceed the scope of this article.

Bony bridging of the peripheral callus is indicative of successful fracture healing, and is taken as the point at which the patient can resume loading of the bone. Depending on the type of fracture, the fixation method and the age of the patient, this point is usually reached after 8–16 weeks.[71]

Hypertrophic non-union occurs when fibrocartilaginous tissue persists between both bony wedges over many months (usually ≥9 months) and bony bridging does not happen. In otherwise healthy patients, non-union most often occurs if the fracture is associated with considerable soft tissue damage,[4] is not sufficiently stabilized or a large fracture gap remains after fragment reduction.[4,22]

Metaphyseal and epiphyseal fracture healing

Although many clinical fractures involve metaphyseal bone, only a limited number of experimental studies have analyzed trabecular bone healing. Such bone healing can be assumed to follow the same phases of inflammation, repair and remodeling; however, no specific molecular studies have been performed to date.

In contrast to diaphyseal fracture repair, trabecular bone healing in the metaphyseal and epiphyseal regions occurs with limited or no periosteal callus formation.[76,77] During the first days post-fracture, tissue reactions are similar to the diaphyseal healing pattern; necrotic tissue is resorbed, the healing area is hypervascularized, and MSCs are recruited, proliferate and differentiate.[77] Initially, a highly vascularized granulation tissue fills the fracture gaps, which is gradually replaced by woven bone and new trabeculae (Figure 3b).[76,77] If the fragments are in contact and are stably fixed, the formation of woven bone and apposition of lamellar bone occurs within 1 week post-fracture,[29] and osteoblastic activity produces new trabeculae in large numbers either side of the fracture (Figure 3c). Existing trabeculae are surrounded by osteoblasts, which progress towards the fracture line while building osteoids—the organic unmineralized bone matrix.[77] New trabeculae are more densely arranged, thus a zone of higher bone density forms parallel to the fracture line (Figure 3c).[78] The new trabeculae are also thicker than normal trabeculae, but are remodeled after healing (3–4 weeks post-fracture) by osteoclasts and osteoblasts.[76,78] After bony bridging of the fracture, vascularization returns to normal levels.[77]

As in diaphyseal bone healing, trabecular bone healing can be delayed depending on fracture gap size and fixation stability.[76] Which tissue develops in the fracture gap depends on local interfragmentary movement and the corresponding interfragmentary strain.[76,79] Under unstable conditions, the early mesenchymal tissue becomes fibrous and cartilaginous (Figure 3c),[76,77,79] and the fibrocartilage and connective tissue can persist. Meanwhile, the new trabeculae create a dense band of bone parallel to the fracture, some external callus formation occurs, and often a non-union ensues.[77] Under successful stabilization, however, metaphyseal bone healing is faster than diaphyseal healing,[77] which might be the result of a number of contributing factors: the trabeculae's large surface area; higher bone formation and mineralization rates;[80] better blood supply; and a thicker periosteum with greater cellularity.[81]

Remodeling phase

Once the diaphyseal fracture gap is filled by new bone, resorption of the periosteal callus begins with osteoclastic activity at the outer surface. Woven bone formed in the cortical fracture gap is remodeled to lamellar bone by osteon formation, similar to primary bone healing. Levels of most inflammatory cytokines are now reduced, although some—in particular IL-1, TNF and BMP-2—are still highly expressed.[13,62] In contrast with hypervascularization of the fracture zone during the repair phase, vascularization during remodeling is reduced to prefracture levels.[32] Remodeling and resorption of the periosteal and medullary calluses leads to reshaping of a diaphyseal bone, which takes approximately 5–8 weeks in rats and can take years in humans. The final outcome is fully-loadable reconstructed bone.

Bone healing and excess inflammation

Systemic inflammation

Chronic

The close relationship between systemic immunity and bone architecture is illustrated in chronic inflammatory diseases such as rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD), diabetes mellitus and systemic lupus erythematosus (SLE). These diseases display systemic inflammation that is closely associated with bone loss and secondary osteoporosis, and, consequently, increased fracture risk.[82] Many of the proinflammatory cytokines (IL-1, IL-6, TNF) abundant in these diseases strongly induce osteoclastogenesis through stimulation of osteoblasts or activated T cells to release RANKL, which interacts with receptor activator of nuclear factor κB (RANK) on the osteoclast surface leading to osteoclast activation.[82,83] The result is an imbalance between bone resorption and formation, disturbing the remodeling process.

Whereas the influence of chronic systemic inflammatory conditions on bone remodeling is well characterized, few clinical studies have investigated the effect of such conditions on fracture healing. Diabetes mellitus is associated with systemic inflammation,[84] mainly attributable to the autoimmune reaction targeting islet beta cells in the case of type 1 diabetes mellitus,[84] whereas in type 2 diabetes mellitus the inflammation is obesity-related and originates directly in adipose tissue.[85,86] Clinical studies have shown impaired fracture healing in patients with diabetes mellitus,[87] and the results of experiments in animal models suggest that disrupted repair is at least partly caused by inflammatory mediators. In particular, TNF was shown to be associated with increased chondrocyte apoptosis, premature loss of cartilage and enhanced osteoclast formation during diabetic fracture healing.[88–90] In a retrospective study, fracture healing in patients with RA was associated with higher complication rates, including non-unions, but the underlying molecular mechanisms remain unknown.[91] Immunoglobulin and complement deposition was found at the site of a non-healing fracture in a patient with SLE;[92] the authors of this study concluded that disease-related autoantibodies inhibited bone cell differentiation, resulting in non-union.[92]

Acute

In comparison with chronic inflammatory diseases, the influence of acute systemic inflammations (polytrauma or sepsis) on fracture healing has been better characterized. In this context, activation of a specific immune cell types (PMNs or macrophages) has considerable importance. Systemic activation of PMNs was reported to impair rodent fracture healing.[55] The detrimental effect of PMNs on bone healing during systemic inflammation was confirmed by the observation of enhanced fracture repair in animals made systemically neutropenic.[93] Furthermore, longer fracture healing times were observed in patients with polytrauma[4,5] possibly as a result of the complex post-traumatic systemic inflammatory response, characterized by rapid proinflammatory cytokine and chemokine release, complement activation, and overactivation of PMNs.[94]

In 2011, we reported that systemic inflammation induced by severe thoracic trauma considerably impaired femoral fracture healing in rats, potentially owing to the influence of systemic inflammation on local inflammatory and early regenerative processes at the fracture site.[95] By contrast, immunomodulation of the post-traumatic inflammatory response using a C5a anaphylatoxin chemotactic receptor antagonist markedly reduced the negative effect of thoracic trauma on fracture healing in this model.[96] Ongoing studies in our institute are investigating the underlying cellular and molecular mechanisms of impaired fracture healing after systemic inflammation induced by severe trauma.

Further evidence for perturbation of fracture healing processes by acute systemic inflammation comes from an experimental rat sepsis model of endotoxinemia induced by systemic treatment with lipopolysaccharide (LPS). In this model, hypertrophic and immature fracture calli with decreased biomechanical properties were found.[97] The authors of this study speculated that osseous healing might be disrupted as a result of decreased BMP-2 production by macrophages,[97] as these cells lost their ability to synthesize BMP-2 after LPS treatment *in vitro*.[98] On the other hand, systemic macrophage activation using semi-soluble aminated glucan did not affect fracture healing outcome in

Box 1 | Factors that might enhance or inhibit bone healing

Factors that enhance bone healing
- Growth factors and hormones: bone morphogenetic proteins;[110,111] parathyroid hormone;[111,112] vascular endothelial growth factor;[113] platelet-derived growth factor;[114] insulin-like growth factor;[115] growth hormone;[116,117] fibroblast growth factor;[111,112] transforming growth factor-β[115]
- Osteogenic cells: mesenchymal stem cells[65]
- Osteoconductive scaffolds:[112] autograft; allograft; demineralised bone matrix; ceramics
- Mechanical environment: improved fixation stability;[22,95,118–120] low-intensity pulsed ultrasonography[121]
- Others: anti-dickkopf-1 antibodies;[122] anti-sclerostin antibodies;[123] prostaglandin E2 receptor agonists;[124] vitamins C, D and E;[125–127] thrombin-related peptide (TP508)[105]

Factors that inhibit bone healing
- Significant factors according to Bhandari and colleagues:[4] severe open fractures; transverse fractures; large fracture gaps

rats;[99] therefore, the role of macrophages in bone healing after systemic inflammation requires further elucidation. The interaction between systemic immunity and the fracture healing process is highly complex, and we are just beginning to understand this relationship.

Local inflammation
As we have outlined above, inflammation is an important factor during bone healing, with molecular factors and immune cells appearing locally at the fracture site in a distinct spatial and temporal manner. Disturbances to this finely tuned sequence of events leads to impaired fracture healing, as demonstrated in certain gene knockout animal models (such as IL-6 and TNF deficient mice).[100–102]

Evidence suggests that local biomechanical conditions within the fracture zone influence the early inflammatory phase of bone healing. In a sheep bone-healing model, in which healing was mechanically impaired through flexible fixation, the early fracture hematoma and the bone marrow in close proximity to the fracture gap displayed more pronounced inflammation, characterized by a considerably increased abundance of cytotoxic T cells and other leukocytes compared with more rigid fracture fixation.[59] In the impaired healing group the hecharacterized by a considerably increased abundance of cytotoxic T cells and other leukocytes A prolonged inflammatory phase was observed in the impaired healing group.[59] A process that might be driven by cytokines released by activated cytotoxic T cells that can prolong the presence of proinflammatory M1 macrophages, possibly by delaying their differentiation into the more anti-inflammatory and proangiogenic M2 macrophages. This theory is supported by the observation of impaired fracture healing after local stimulation of macrophage to secrete—predominantly proinflammatory—cytokines.[99]

Interesting insights into the effect of local inflammation on bone healing come from a rabbit model of inflammatory arthritis—a disease characterized by a strong juxta-articular osteopenia.[103,104] Surprisingly, the fracture healing process was not disturbed by the inflammatory arthritis compared to healthy joints.[103,104] Furthermore, the authors found greatly increased new bone formation in intact bone adjacent to the fracture healing zone.[103,104] This finding indicates that fracture repair processes can override the bone loss caused by inflammatory arthritis. Therefore, a local proinflammatory milieu does not necessarily lead to impaired bone healing, a conclusion supported by evidence from a number of studies. For example, local application of thrombin peptide (TP508) increased the early expression of a wide range of proinflammatory mediators (such as IL-6) at the fracture site and enhanced healing.[105] Furthermore, Hankemeier *et al.*[106] observed increased macrophage recruitment, but shorter residency time, in fracture calli stabilized by rigid fixation compared with less stable fixation. In addition, decreased macrophage recruitment at the fracture site has been shown to impair vascularization, reduce bone formation, disturb osteoclastic functionality, and, consequently, delay fracture healing.[54] These data imply that the inflammatory response of macrophages at the fracture site is indispensable during at least some periods of bone healing.

The contribution of the adaptive immune system to the fracture healing cascade was investigated by Toben *et al.*[107] using recombination activating gene 1 knockout (*Rag1*$^{-/-}$) mice, which specifically lack T cells and B cells. Interestingly, fracture healing was improved in the absence of T cells and B cells, indicating that the activation of the adaptive immune response might have a negative effect on bone regeneration.[107]

In conclusion, a smooth transition between 'good' and 'bad' inflammation at the fracture site seems to exist, which depends on the quantitative, qualitative and temporal composition of the fracture callus. The consequence of inflammation on fracture healing outcome remains unclear at present. Future studies should aim to better characterize this phase of fracture repair and its relationship to the healing outcome. This knowledge might enable the development of strategies to prevent impaired bone healing in patients with compromised immune systems.

Factors that affect fracture healing
Giannoudis *et al.*[108] proposed the 'diamond concept' for treatment of fractures, which takes four main factors into account (growth factors, osteoconductive scaffolds, mesenchymal stem cells and the mechanical environment), consideration of which might improve fracture healing (Box 1). Many risk factors for impaired fracture healing exist: type of injury (fracture geometry, degree of open injury, mechanism of injury); fracture treatment (type of fixation, size of fracture gaps); gender; age; comorbidities (diabetes mellitus, malnutrition, peripheral vascular disease, hypothyroidism, polytrauma); medications (NSAIDs, corticosteroids, antibiotics, anticoagulants); smoking; and alcohol consumption.[4,109] Among these risk factors, Bhandari *et al.*[4] determined important predictors of reoperation following operative management

of fractures of the tibial shaft (Box 1). In keeping with data from previously published studies,[109] Bhandari and colleagues[4] found that some risk factors significantly correlated with a high rate of reoperation in the univariate statistical analysis. However, in a multivariate statistical analysis, which controls for interdependent effects between the various factors, only three prognostic risk factors for reoperation reached significance:[4] severe open fractures,[44] transverse fractures,[4] and large postoperative fracture gaps (lack of cortical continuity).[22]

Conclusions

That fractures should be sufficiently stabilized and the local blood supply of the traumatized tissue saved by minimal invasive treatment methods is generally accepted. The optimal healing conditions are, however, dependent on various factors, such as trauma severity, type and location of the fracture, and the presence of additional disease. Systemic inflammation observed in patients with RA, diabetes mellitus or multiple traumata, for example, seems to impair fracture healing. Evidence suggests that systemic inflammation and local physiological inflammation interact during the early healing phase of fracture repair, and that local inflammation is affected by the biomechanical environment in the fracture healing zone. The molecular events underlying these interactions, however, are not fully understood and require further research.

Review criteria

We searched for original articles focusing on fracture healing published between 1960 and 2011 in MEDLINE and PubMed and in personal collections of references. The search terms used were "fracture healing", "local inflammation", "systemic inflammation", "immune cells", "neutrophils", "macrophages", "lymphocytes", "fracture hematoma", "cytokines", "secondary osteoporosis", "rheumatoid arthritis", "diabetes", "fixation stability" and "angiogenesis" alone and in various combinations. All articles identified were English-language, full-text papers, except three articles in German with English abstracts. We also searched the reference lists of identified articles for further relevant papers.

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Author contributions

The authors contributed equally to all stages of the preparation of this manuscript.