Capítulo 15

Bone Healing: Biological Basis and Factors Influencing Fracture Repair

Consolidação Óssea: Bases Biológicas e Fatores que Influenciam o Reparo das Fraturas

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Abstract: Bone healing is a highly coordinated biological process that restores the structural integrity, mechanical strength, and functional capacity of fractured bone. Unlike most connective tissues, bone possesses a remarkable regenerative capacity, allowing complete restoration of its original architecture through the interaction of inflammatory, cellular, vascular, molecular, and biomechanical mechanisms. Fracture healing progresses through overlapping phases, including hematoma formation, inflammation, angiogenesis, soft callus formation, endochondral ossification, and bone remodeling. These events are regulated by numerous signaling molecules, including bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and the RANK/RANKL/osteoprotegerin pathway. Successful fracture repair depends not only on adequate mechanical stability but also on preservation of vascular supply, periosteal integrity, soft tissues, and systemic metabolic homeostasis. Several biological and systemic factors—including diabetes mellitus, nutritional deficiencies, aging, smoking, impaired vascularization, and endocrine disorders—may compromise bone regeneration and increase the risk of delayed union or nonunion. In addition, insulin signaling, glucose metabolism, and appropriate nutritional support play essential roles in sustaining the high metabolic demands of osteogenic cells during tissue regeneration. This review summarizes the cellular and molecular mechanisms involved in fracture healing and discusses the principal biological factors influencing successful bone repair, providing an integrated overview of the physiological basis of skeletal regeneration and its clinical implications.

Keywords: bone healing; fracture repair; osteogenesis; angiogenesis; bone remodeling; fracture union.

Resumo: A cicatrização óssea é um processo biológico altamente coordenado que restaura a integridade estrutural, a resistência mecânica e a capacidade funcional do osso fraturado. Diferentemente da maioria dos tecidos conjuntivos, o osso possui uma notável capacidade regenerativa, permitindo a restauração completa de sua arquitetura original por meio da interação entre mecanismos inflamatórios, celulares, vasculares, moleculares e biomecânicos. A consolidação da fratura progride por fases sobrepostas, incluindo formação do hematoma, inflamação, angiogênese, formação de calo mole, ossificação endocondral e remodelação óssea. Esses eventos são regulados por inúmeras moléculas sinalizadoras, incluindo proteínas morfogenéticas ósseas (BMPs), fator de crescimento endotelial vascular (VEGF),

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fator de crescimento derivado de plaquetas (PDGF), fator de crescimento transformador beta (TGF-β) e a via RANK/RANKL/osteoprotegerina. O reparo adequado da fratura depende não apenas da estabilidade mecânica adequada, mas também da preservação do suprimento vascular, da integridade do periósteo, dos tecidos moles e da homeostase metabólica sistêmica. Diversos fatores biológicos e sistêmicos — incluindo diabetes mellitus, deficiências nutricionais, envelhecimento, tabagismo, comprometimento da vascularização e distúrbios endócrinos — podem prejudicar a regeneração óssea e aumentar o risco de consolidação tardia ou não união. Além disso, a sinalização da insulina, o metabolismo da glicose e o suporte nutricional adequado desempenham papéis essenciais na manutenção da alta demanda metabólica das células osteogênicas durante a regeneração tecidual. Esta revisão sintetiza os mecanismos celulares e moleculares envolvidos na cicatrização óssea e discute os principais fatores biológicos que influenciam o sucesso do reparo ósseo, fornecendo uma visão integrada da base fisiológica da regeneração esquelética e suas implicações clínicas.

Palavras-chave: cicatrização óssea; reparo de fraturas; osteogênese; angiogênese; remodelação óssea; consolidação de fratura.

INTRODUCTION

Bone fractures are characterized by the disruption of the structural continuity of bone tissue and are generally accompanied by varying degrees of injury to the surrounding soft tissues (Sheen, Mabrouk, & Garla, 2026). They represent a major cause of morbidity and functional disability worldwide, making a comprehensive understanding of the biological mechanisms underlying bone repair essential for clinical practice.

Bone healing is a highly coordinated and dynamic biological process that restores the structural integrity, mechanical strength, and functional capacity of fractured bone. Unlike most connective tissues, bone possesses a remarkable regenerative potential, allowing complete restoration of its original architecture rather than the formation of fibrotic scar tissues. This regenerative capacity results from the complex interaction of cellular, molecular, vascular, and biomechanical factors that act sequentially throughout the healing process. Following a fracture, a cascade of overlapping biological events is initiated, including hematoma formation, inflammation, angiogenesis, recruitment and differentiation of mesenchymal progenitor cells, synthesis of cartilaginous and osseous extracellular matrix, mineralization, and subsequent bone remodeling. Each stage is tightly regulated by signaling molecules, growth factors, cytokines, and mechanical stimuli, which collectively determine the quality and rate of fracture repair.

Successful bone healing depends not only on the intrinsic biological capacity of bone tissue but also on several local and systemic factors. Adequate vascularization, preservation of the periosteum and surrounding soft tissues, mechanical stability, nutritional status, endocrine regulation, age, and the presence of metabolic disorders all influence the healing outcome. Conversely, impaired vascular supply, excessive instability, infection, smoking, diabetes mellitus, and certain medications may delay union or lead to nonunion. A thorough understanding

of the biological principles governing fracture healing is fundamental for interpreting the progression of bone repair, identifying factors that compromise regeneration, and selecting the most appropriate therapeutic strategies. Advances in molecular biology, tissue engineering, regenerative medicine, and orthopedic surgery have substantially expanded our understanding of bone healing mechanisms, creating new opportunities to improve clinical outcomes and optimize fracture repair.

STRUCTURE AND PHYSIOLOGY OF BONE TISSUE

Bone is a specialized connective tissue composed of two major components: an inorganic mineralized matrix, which provides rigidity and compressive strength, and an organic matrix, which confers elasticity and tensile resistance. The interaction between these two components enables bone to withstand mechanical loads while maintaining sufficient flexibility to prevent fracture under physiological conditions.

The inorganic matrix accounts for approximately 70% of bone mass and consists predominantly of calcium and phosphate salts organized as hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$). These crystals are deposited within the organic matrix, particularly along collagen fibers, providing the tissue with its characteristic hardness and resistance to compression. A small fraction of bone calcium remains in a non-crystalline form, constituting a readily exchangeable reservoir that contributes to the maintenance of extracellular calcium homeostasis (Hall; Hall, 2023). The organic matrix represents approximately 30% of bone composition and is composed primarily of type I collagen fibers, together with proteoglycans, glycoproteins, and several non-collagenous proteins. Type I collagen is responsible for the tensile strength and flexibility of bone tissue, whereas proteoglycans and glycoproteins contribute to extracellular matrix organization, mineral deposition, and cell-matrix interactions. Bone cells, including osteoblasts, osteocytes, and osteoclasts, act in a coordinated manner to regulate bone formation, maintenance, and remodeling (Junqueira; Carneiro, 2023).

From a structural perspective, bone tissue is classified into two distinct types: cortical (compact) bone and trabecular (cancellous or spongy) bone. Although both possess the same basic cellular and extracellular composition, they differ considerably in their architecture, density, mechanical properties, anatomical distribution, and physiological functions (Hall; Hall, 2023). Cortical bone, also referred to as compact bone, forms the dense outer shell of all bones and accounts for approximately 80% of total skeletal mass. It is characterized by low porosity and a highly organized lamellar architecture arranged into cylindrical structural units known as osteons, or Haversian systems. This organization provides remarkable resistance to compression, bending, and torsional forces, allowing cortical bone to perform essential mechanical functions such as weight bearing, locomotion, and protection of internal organs (Junqueira; Carneiro, 2023).

In contrast, trabecular bone is located predominantly within the interior of bones, particularly in the epiphyses of long bones, vertebral bodies, the pelvis, and

flat bones. It consists of an interconnected three-dimensional network of trabeculae that delimit marrow-filled spaces. This porous architecture substantially reduces skeletal weight while efficiently distributing mechanical loads and absorbing impact forces. Beyond its mechanical role, trabecular bone exhibits intense metabolic activity. Owing to its large surface area and rich vascular supply, it undergoes substantially higher rates of bone formation and resorption than cortical bone. Consequently, bone remodeling occurs more rapidly within trabecular compartments, making them particularly responsive to hormonal regulation, metabolic alterations, aging, and pathological conditions affecting bone metabolism, such as osteoporosis (Hall; Hall, 2023).

Bone tissue is continuously renewed throughout life through the process of bone remodeling, in which old or damaged bone is resorbed by osteoclasts and replaced by newly formed bone synthesized by osteoblasts. This tightly regulated process preserves skeletal integrity, repairs microscopic damage, adapts bone architecture to mechanical loading, and plays a critical role in maintaining systemic calcium and phosphate homeostasis. The balance between bone formation and resorption is regulated by mechanical stimuli, endocrine factors, cytokines, and multiple signaling pathways that coordinate the activity of bone cells.

BONE CELL TYPES

Bone tissue is composed of four major cell populations that play distinct yet highly coordinated roles in skeletal development, maintenance, remodeling, and fracture repair: osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts. The dynamic balance among these cell types is essential for preserving bone homeostasis and ensuring successful bone regeneration following injury.

Osteoprogenitor Cells: Osteoprogenitor cells are mesenchymal stem cells committed to the osteogenic lineage and represent the primary source of osteoblasts. They are located predominantly within the cambium layer of the periosteum, the endosteum, and other bone-lining surfaces, where they normally remain in a quiescent state.

During skeletal growth, physiological remodeling, or following bone injury, these cells become activated and proliferate through mitosis before differentiating into mature osteoblasts. Osteogenic differentiation is tightly regulated by several transcription factors, including Runt-related transcription factor 2 (RUNX2), SRY-box transcription factor 9 (SOX9), and Osterix (SP7), together with signaling molecules such as bone morphogenetic proteins (BMPs), which are indispensable for osteogenesis and skeletal development (Junqueira; Carneiro, 2023). Because of their high proliferative and differentiation potential, osteoprogenitor cells play a central role in fracture healing and bone regeneration, serving as the principal cellular reservoir responsible for new bone formation.

Osteoblasts: Osteoblasts are the primary bone-forming cells and are responsible for synthesizing the organic extracellular matrix of bone. These

metabolically active mononuclear cells are located along bone surfaces, where they form a continuous layer during periods of active bone formation. Their principal function is the synthesis and secretion of osteoid, the unmineralized organic matrix composed predominantly of type I collagen, together with proteoglycans, glycoproteins, and several non-collagenous proteins. Osteoblasts also produce enzymes such as alkaline phosphatase, which promotes matrix mineralization through the deposition of hydroxyapatite crystals. In addition to matrix production, osteoblasts secrete numerous growth factors that regulate bone metabolism and tissue repair, including insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and basic fibroblast growth factor (bFGF). These molecules stimulate cell proliferation, extracellular matrix synthesis, angiogenesis, and communication among bone cells, thereby coordinating the healing response (Mullis; Gaski, 2020).

Osteoblasts also play a fundamental regulatory role in bone remodeling by controlling osteoclast differentiation through the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) and its decoy receptor osteoprotegerin (OPG). The balance between RANKL and OPG determines osteoclast formation and, consequently, the rate of bone resorption. Once matrix synthesis is completed, osteoblasts undergo one of three possible fates: apoptosis, transformation into quiescent bone-lining cells, or entrapment within the mineralized matrix, where they differentiate into osteocytes (Junqueira; Carneiro, 2023).

Osteocytes: Osteocytes are the most abundant cells in bone tissue, accounting for approximately 90–95% of all bone cells. They represent the terminally differentiated form of osteoblasts and become embedded within the mineralized bone matrix inside microscopic cavities known as lacunae. Each osteocyte extends numerous cytoplasmic processes through narrow channels called canaliculi, establishing an extensive three-dimensional communication network interconnected by gap junctions. This network allows the exchange of ions, nutrients, metabolites, and signaling molecules between neighboring osteocytes and cells located on bone surfaces (Junqueira; Carneiro, 2023). Although osteocytes exhibit limited synthetic activity, they are the principal mechanosensory cells of bone. They continuously monitor mechanical loading and detect alterations in fluid flow within the lacunar-canalicular system. Mechanical signals are converted into biochemical responses that regulate both osteoblast and osteoclast activity, thereby coordinating adaptive bone remodeling according to functional demands.

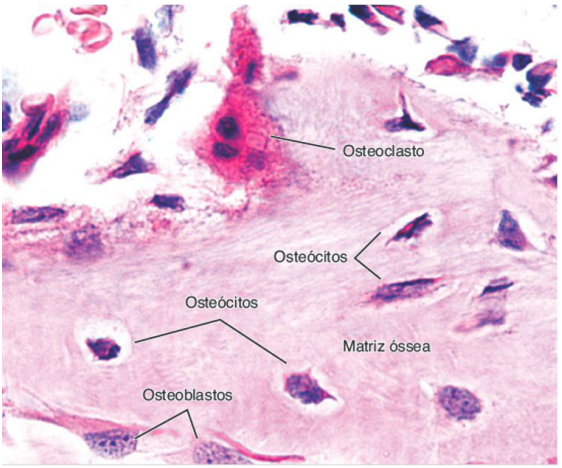
Osteocytes synthesize several biologically active molecules involved in bone metabolism, including osteocalcin, osteopontin, fibroblast growth factor-23 (FGF-23), and sclerostin. Among these, sclerostin is particularly important because it inhibits osteoblast differentiation and suppresses bone formation by antagonizing the Wnt/β-catenin signaling pathway, one of the principal regulators of osteogenesis (Junqueira & Carneiro, 2023). Consequently, osteocytes function not only as mechanosensors but also as endocrine-like regulators that coordinate mineral metabolism and skeletal homeostasis.

Osteoclasts: Osteoclasts are large multinucleated cells specialized in bone resorption. Unlike osteoblasts and osteocytes, which originate from mesenchymal stem cells, osteoclasts arise from the hematopoietic monocyte/macrophage lineage through the fusion of multiple precursor cells derived from the bone marrow. These highly polarized cells are located on bone surfaces undergoing active remodeling. During bone resorption, osteoclasts adhere tightly to the mineralized matrix, forming a specialized sealing zone that isolates the resorption compartment from the surrounding extracellular environment. Within this compartment, osteoclasts secrete hydrochloric acid through proton pumps, dissolving hydroxyapatite crystals, while simultaneously releasing lysosomal enzymes such as cathepsin K and matrix metalloproteinases (MMPs) that degrade the organic matrix, particularly type I collagen.

Osteoclast differentiation and activation depend primarily on two essential signaling molecules: macrophage colony-stimulating factor (M-CSF) and RANKL, both produced by osteoblasts and osteocytes. Binding of RANKL to its receptor expressed on osteoclast precursors activates intracellular signaling pathways that promote osteoclast maturation, survival, and bone-resorptive activity. Conversely, osteoprotegerin (OPG) inhibits this interaction by acting as a soluble decoy receptor, thereby suppressing osteoclastogenesis (Mullis; Gaski, 2020).

Bone resorption performed by osteoclasts is indispensable for physiological bone remodeling, fracture repair, calcium homeostasis, and the replacement of damaged or aged bone tissue. However, excessive osteoclastic activity contributes to pathological conditions such as osteoporosis, inflammatory bone destruction, and skeletal metastases. The balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption is therefore fundamental for maintaining skeletal integrity and ensuring successful fracture healing (Junqueira; Carneiro, 2023).

Figure 1 - Histological section of bone tissue showing osteoblasts, osteocytes, osteoclasts, and bone matrix.



Fonte: Junqueira; Carneiro (2023, p. 145).

BIOLOGICAL PROCESS OF FRACTURE HEALING

Fracture healing is a dynamic, highly coordinated biological process that restores the structural integrity and mechanical properties of injured bone. Unlike the repair of most connective tissues, bone healing culminates in true tissue regeneration rather than scar formation. This regenerative process depends on the synchronized interaction of inflammatory cells, mesenchymal stem cells, osteogenic cells, vascular elements, growth factors, cytokines, and mechanical stimuli. Traditionally, fracture healing is divided into four overlapping biological phases: (1) hematoma formation and inflammatory response; (2) soft callus formation; (3) hard callus formation through endochondral ossification; and (4) bone remodeling (Bucholz *et al.,* 2013).

Hematoma Formation and Inflammatory Response: Immediately after a fracture occurs, disruption of the periosteal, cortical, and medullary blood vessels results in local hemorrhage and the rapid formation of a fracture hematoma. Rather than representing a simple blood clot, this hematoma constitutes a highly specialized biological environment that initiates the healing cascade. The fracture hematoma contains platelets, inflammatory cells, fibrin, cytokines, chemokines, and numerous growth factors that establish the molecular signals necessary for tissue regeneration. Temporary interruption of blood flow creates a hypoxic microenvironment that activates multiple intracellular signaling pathways involved in repair. Within the first hours after injury, neutrophils migrate to the fracture site, followed by macrophages and lymphocytes. These inflammatory cells remove necrotic tissue, eliminate cellular debris, and release cytokines that orchestrate subsequent stages of healing.

Among the principal inflammatory mediators are interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which stimulate recruitment of mesenchymal stem cells, enhance angiogenesis, and initiate osteogenic differentiation (Marsell & Einhorn, 2011). Platelets trapped within the hematoma release large quantities of platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), both of which stimulate chemotaxis, cellular proliferation, extracellular matrix synthesis, and recruitment of osteoprogenitor cells required for bone regeneration (Skinner; McMahon, 2015).

Angiogenesis and Recruitment of Mesenchymal Stem Cells: One of the earliest and most critical events in fracture healing is the restoration of local blood supply through angiogenesis. Because fracture-induced vascular disruption creates a hypoxic environment, stabilization of hypoxia-inducible factor-1 alpha (HIF-1α) stimulates expression of vascular endothelial growth factor (VEGF), the principal regulator of neovascularization. VEGF promotes endothelial cell proliferation, migration, and organization into newly formed capillaries that progressively re-establish blood flow within the fracture site. Restoration of vascularization supplies oxygen, glucose, amino acids, minerals, and additional progenitor cells required for tissue regeneration.

Newly formed blood vessels also serve as migration pathways for mesenchymal stem cells derived from the periosteum, bone marrow, surrounding soft tissues, and systemic circulation. The periosteum represents the principal source of osteogenic

progenitor cells because its cambium layer contains abundant osteoprogenitor cells capable of rapid proliferation following injury (Junqueira; Carneiro, 2023).

Formation of the Soft Callus: As inflammation subsides, mesenchymal stem cells begin to proliferate and differentiate according to the biological and mechanical conditions present within the fracture environment. Under conditions of reduced oxygen tension, increased interfragmentary motion, and relatively acidic pH, mesenchymal cells preferentially differentiate into chondrocytes, initiating chondrogenesis.

These chondrocytes synthesize an extracellular matrix rich in type II collagen, proteoglycans, and glycosaminoglycans, producing a fibrocartilaginous structure known as the soft callus. Although mechanically weak, the soft callus stabilizes fracture fragments, decreases local motion, and provides a biological scaffold for subsequent bone formation.

Differentiation of mesenchymal cells into osteoblasts and chondrocytes is regulated primarily by bone morphogenetic proteins (BMPs), particularly BMP-2, BMP-4, and BMP-7, which activate transcription factors including RUNX2 and Osterix, thereby promoting osteogenesis.

Hard Callus Formation (Endochondral Ossification): As angiogenesis progresses and oxygen availability increases, the fibrocartilaginous callus gradually undergoes mineralization and is replaced by woven bone through endochondral ossification. Hypertrophic chondrocytes within the soft callus begin expressing type X collagen, alkaline phosphatase, and VEGF, promoting calcification of the cartilaginous matrix while simultaneously attracting additional blood vessels.

The mineralized cartilage is progressively resorbed by osteoclasts and chondroclasts, creating a scaffold upon which osteoblasts deposit osteoid that subsequently mineralizes to form immature woven bone. This stage produces the hard callus, which bridges the fracture gap and restores substantial mechanical stability. Unlike mature lamellar bone, woven bone is characterized by randomly oriented collagen fibers and lower mechanical strength. Nevertheless, it provides sufficient structural support to permit progressive functional loading during healing.

Bone Remodeling: The final stage of fracture healing consists of prolonged bone remodeling, during which immature woven bone is gradually replaced by mature lamellar bone. This process is coordinated through the balanced activities of osteoclasts and osteoblasts operating within basic multicellular units (BMUs). Osteoclasts selectively resorb woven bone while osteoblasts synthesize organized lamellar bone following the principal mechanical stress lines described by Wolff's Law. Throughout remodeling, the medullary canal is re-established, cortical thickness is restored, and bone architecture progressively returns to its pre-injury configuration. Depending on patient age, fracture location, mechanical loading, and biological conditions, remodeling may continue for several months or even years after clinical union has occurred.

Cellular and Molecular Regulation of Fracture Healing

Fracture healing is controlled by an intricate signaling network involving inflammatory cytokines, growth factors, transcription factors, and mechanotransduction pathways. Among the most important regulators are: BMPs (Bone Morphogenetic Proteins): induce osteogenic differentiation; VEGF (Vascular Endothelial Growth Factor): stimulates angiogenesis; PDGF (Platelet-Derived Growth Factor): promotes chemotaxis and cell proliferation; TGF-β (Transforming Growth Factor-β): regulates extracellular matrix synthesis and cellular differentiation; FGFs (Fibroblast Growth Factors): stimulate proliferation of mesenchymal cells and endothelial cells; RUNX2 and Osterix (SP7): master transcription factors controlling osteoblast differentiation; RANK/RANKL/OPG signaling: regulates osteoclast formation and bone remodeling; Wnt/β-catenin signaling: promotes osteoblast differentiation and bone formation while inhibiting bone resorption.

The coordinated interaction among these molecular pathways ensures proper progression through each stage of fracture healing. Disruption of any of these mechanisms, whether due to impaired vascularization, infection, metabolic disorders, excessive mechanical instability, or systemic disease, may compromise bone repair and lead to delayed union or nonunion.

TYPES OF FRACTURE HEALING: Fracture healing occurs through two distinct biological mechanisms: primary (direct) bone healing and secondary (indirect) bone healing. Although both ultimately restore skeletal continuity, they differ substantially in their biological processes, mechanical requirements, and clinical indications. Secondary fracture healing is by far the most common mechanism observed in clinical practice and occurs under conditions of relative mechanical stability. This process is characterized by sequential callus formation and involves both intramembranous and endochondral ossification. Conversely, primary fracture healing requires absolute mechanical stability, typically achieved through rigid internal fixation using compression plates or lag screws. Under these conditions, fracture repair proceeds without visible callus formation because bone remodeling occurs directly across the fracture line through osteonal reconstruction (Skinner & McMahon, 2015).

Primary (Direct) Bone Healing: Primary bone healing occurs only when fracture fragments are anatomically reduced, and interfragmentary strain is extremely low, generally below 2%. These conditions permit direct reconstruction of cortical bone without the intermediate formation of fibrocartilage or woven bone. Two biological mechanisms contribute to direct healing:

Contact Healing: When the fracture gap is less than approximately 0.01 mm, osteoclasts form cutting cones that cross the fracture line. These cutting cones are followed by capillary sprouts and osteoblasts, which deposit new lamellar bone directly behind the advancing osteoclasts. This process resembles physiological cortical bone remodeling through reconstruction of new Haversian systems (osteons). As healing progresses, vascular continuity is restored, and newly formed osteons bridge the fracture site, producing complete cortical reconstruction without external callus formation.

Gap Healing: When rigid fixation is achieved but a small fracture gap (typically less than 1 mm) persists, healing occurs initially through deposition of woven bone within the gap. This immature bone is subsequently remodeled into organized lamellar bone through osteonal remodeling. Although gap healing involves an initial phase of woven bone formation, it remains classified as primary healing because fracture stability prevents the development of a cartilaginous callus. Primary healing is commonly observed following compression plating, interfragmentary screw fixation, and certain joint reconstruction procedures, where restoration of anatomical alignment is essential.

Secondary (Indirect) Bone Healing: Secondary fracture healing represents the physiological mechanism responsible for the repair of most fractures. Unlike primary healing, it occurs under conditions of relative stability, where controlled interfragmentary motion stimulates biological repair.

This mechanism proceeds through four overlapping stages: Hematoma formation and inflammation; Soft callus formation; Hard callus formation; Bone remodeling. During this process, both intramembranous ossification and endochondral ossification contribute to bone regeneration. Intramembranous bone formation occurs mainly beneath the periosteum, whereas endochondral ossification predominates within the fracture gap, where cartilage is progressively replaced by bone. The temporary fibrocartilaginous callus functions as a biological bridge that stabilizes fracture fragments while allowing vascular invasion and progressive mineralization.

Mechanical Regulation of Fracture Healing: Mechanical stability is one of the principal determinants of the biological pathway followed during fracture healing. According to the Interfragmentary Strain Theory proposed by Perren, tissue differentiation depends largely on the amount of strain experienced at the fracture site.

Different tissues exhibit varying levels of tolerance to mechanical deformation. Granulation tissue shows the highest strain tolerance, withstanding up to approximately 100% strain, which reflects its highly cellular and immature nature during the tissue repair process. Fibrocartilage has an intermediate tolerance, sustaining about 10–15% strain due to its more organized extracellular matrix rich in collagen. Immature bone (woven bone) has a lower deformation capacity, tolerating approximately 2–10% strain, as it is still relatively disorganized despite being mineralized. Finally, lamellar bone, the most mature and well-organized bone tissue, exhibits the lowest strain tolerance, withstanding less than 2% deformation, reflecting its high structural rigidity and specialization for mechanical strength. When strain exceeds the tolerance of newly formed tissues, normal bone formation cannot proceed, increasing the risk of delayed union or nonunion. Conversely, moderate mechanical stimulation enhances callus formation and accelerates fracture repair. This concept explains why modern orthopedic fixation techniques frequently seek relative stability rather than absolute rigidity, especially in comminuted fractures.

Clinical Relevance: Understanding the differences between primary and secondary fracture healing is fundamental for selecting the most appropriate

fixation strategy. Rigid fixation favors anatomical reconstruction and primary healing but requires preservation of vascular supply. Relative stability, achieved through techniques such as intramedullary nailing, bridge plating, and external fixation, preserves the biological environment of the fracture and promotes abundant callus formation. Current concepts in orthopedic trauma surgery emphasize the importance of biological fixation, which minimizes soft-tissue injury and periosteal disruption while maintaining sufficient mechanical stability to stimulate natural bone regeneration. Consequently, successful fracture management depends not only on mechanical stabilization but also on preservation of the biological environment required for tissue repair.

ANGIOGENESIS AND VASCULARIZATION: Angiogenesis, defined as the formation of new blood vessels from pre-existing vasculature, is one of the most critical biological events during fracture healing. Successful bone regeneration depends on the rapid restoration of blood supply to the fracture site, ensuring adequate oxygen delivery, nutrient transport, waste removal, and recruitment of progenitor cells required for tissue repair. Consequently, revascularization is considered a prerequisite for normal fracture healing (Bucholz *et al.,* 2013).

Immediately after fracture, disruption of the cortical, periosteal, and medullary blood vessels results in transient ischemia and local hypoxia. Rather than impairing repair, this hypoxic environment serves as an essential biological stimulus that activates multiple molecular pathways involved in vascular regeneration. The principal regulator of this response is hypoxia-inducible factor-1 alpha (HIF-1α). Under hypoxic conditions, HIF-1α accumulates within cells and stimulates the expression of several angiogenic genes, particularly vascular endothelial growth factors (VEGF).

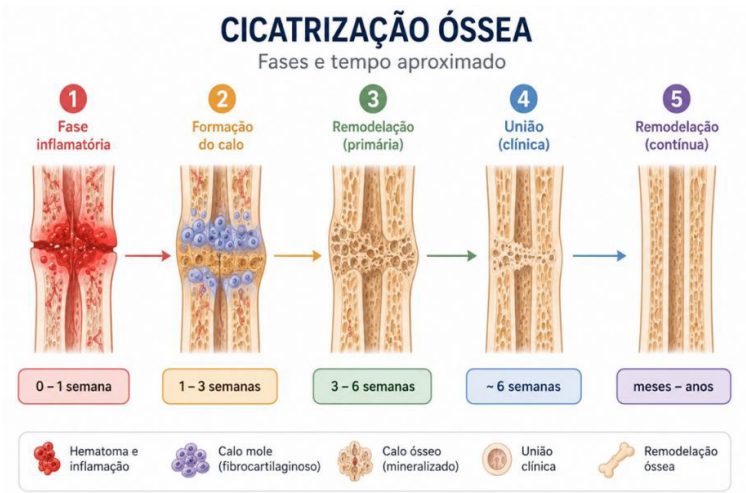
VEGF is recognized as the master regulator of angiogenesis during fracture repair. Produced by inflammatory cells, hypertrophic chondrocytes, osteoblasts, mesenchymal stem cells, and stromal cells located within the fracture microenvironment, VEGF stimulates endothelial cell proliferation, migration, survival, and differentiation, leading to the formation of new capillary networks that progressively restore local circulation (Marsell & Einhorn, 2011). The newly formed blood vessels not only re-establish oxygen and nutrient delivery but also provide migration pathways for inflammatory cells, mesenchymal stem cells, endothelial progenitor cells, osteoblast precursors, and circulating growth factors. Consequently, angiogenesis and osteogenesis are tightly coupled biological processes throughout fracture healing.

Experimental studies have demonstrated that inhibition of VEGF signaling markedly delays fracture repair, reduces callus vascularization, impairs endochondral ossification, and increases the incidence of delayed union and nonunion. Conversely, therapeutic strategies that enhance angiogenesis have shown promising results in accelerating bone regeneration, particularly in cases of compromised healing (Hankenson *et al.,* 2011). As vascular invasion progresses, cartilage within the soft callus becomes progressively mineralized and replaced by woven bone. Thus,

angiogenesis represents the critical transition between the cartilaginous and osseous phases of fracture healing.

Failure of adequate vascularization significantly compromises fracture repair. Inadequate blood supply limits oxygen delivery, decreases osteoblast activity, reduces progenitor cell recruitment, and favors persistence of fibrous tissue instead of bone formation. Among the different forms of nonunion, atrophic nonunion is classically associated with severe vascular insufficiency. It is characterized by minimal callus formation, reduced cellular activity, poor biological potential for healing, and often requires both mechanical stabilization and biological stimulation, including bone grafting or osteoinductive therapies, to achieve successful union (Bucholz *et al.,* 2013). Overall, angiogenesis represents one of the fundamental biological determinants of fracture healing, integrating inflammatory signaling, stem cell recruitment, extracellular matrix formation, and bone regeneration into a coordinated regenerative process.

Figure 2 - Stages of fracture healing.



Fonte: Google.

BIOLOGICAL FACTORS INFLUENCING FRACTURE HEALING: Fracture healing is influenced by numerous biological factors that regulate cellular activity, vascularization, extracellular matrix synthesis, and tissue remodeling. Successful bone regeneration depends on the interaction between local biological conditions and systemic physiological status. Disruption of these factors may impair bone repair and increase the risk of delayed union or nonunion.

Degree of Vascularization: Adequate vascularization is considered one of the most important determinants of successful fracture healing. Blood vessels provide oxygen, glucose, amino acids, minerals, inflammatory cells, mesenchymal stem cells, and growth factors essential for cellular proliferation and differentiation.

Immediately after injury, rupture of periosteal, cortical, and medullary vessels results in formation of the fracture hematoma, which acts as a temporary fibrin-rich

scaffold supporting cell migration and concentrating numerous biological mediators involved in tissue regeneration (Bucholz *et al.,* 2013). The fracture hematoma contains abundant growth factors, including transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and multiple cytokines released by activated platelets. These molecules stimulate chemotaxis, cell proliferation, extracellular matrix synthesis, and angiogenesis.

During the first days following injury, newly formed periosteal vessels constitute the principal blood supply to the fracture site. As healing progresses, medullary circulation gradually becomes re-established and contributes increasingly to bone regeneration. Preservation of vascular integrity during surgical treatment is therefore fundamental. Excessive periosteal stripping, extensive soft-tissue dissection, or disruption of intramedullary blood supply may significantly reduce local vascularity and delay fracture healing. In severe cases of vascular compromise, necrosis of fracture fragments may occur, followed by osteoclastic bone resorption and failure of union (Bucholz *et al.,* 2013).

Soft Tissue Injury: The condition of the surrounding soft tissues exerts a profound influence on fracture healing. Preserved muscles, fascia, periosteum, and connective tissues maintain local vascularization, stabilize the fracture hematoma, provide mesenchymal stem cells, and produce numerous growth factors that contribute to bone regeneration. Conversely, extensive soft tissue damage, commonly observed in high-energy trauma and open fractures, compromises blood supply, disrupts the biological environment, and delays fracture repair.

Mesenchymal stem cells originating from injured soft tissues differentiate into fibroblasts, chondrocytes, and osteoblasts according to local biochemical and mechanical conditions. These cells also produce bone morphogenetic proteins (BMPs), which strongly stimulate osteogenic differentiation and new bone formation (Marsell; Einhorn, 2011). For this reason, modern orthopedic trauma surgery emphasizes the preservation of soft tissues through minimally invasive surgical techniques and biological fixation strategies whenever possible.

METABOLIC SUPPORT FOR BONE REGENERATION: Successful bone regeneration requires an adequate metabolic environment capable of sustaining the intense cellular activity associated with fracture healing. Throughout the repair process, proliferating and differentiating cells exhibit high metabolic demands, requiring a continuous supply of oxygen, energy substrates, amino acids, minerals, vitamins, and growth factors. Consequently, tissue oxygenation and nutrient availability are essential determinants of successful bone repair.

Bone healing is an energy-intensive process involving inflammatory cell activation, mesenchymal stem cell proliferation, extracellular matrix synthesis, angiogenesis, mineralization, and continuous tissue remodeling. These biological events require substantial amounts of adenosine triphosphate (ATP), which is generated primarily through cellular metabolism of glucose and, under aerobic conditions, mitochondrial oxidative phosphorylation. The maintenance of adequate blood flow is therefore fundamental to support cellular metabolism during fracture repair. In particular, the periosteal circulation plays a crucial role by supplying oxygen

and metabolic substrates to osteogenic cells located within the cambium layer of the periosteum, one of the principal sources of osteoblast precursors involved in bone regeneration.

Among the available metabolic substrates, glucose represents the primary energy source for inflammatory cells, osteoblasts, endothelial cells, and mesenchymal stem cells during the early phases of fracture healing. Cellular glucose uptake is largely regulated by insulin, which promotes glucose transport through insulin-sensitive glucose transporters, thereby maintaining intracellular energy production and supporting anabolic processes required for tissue regeneration.

Beyond its role in glucose metabolism, insulin exerts important anabolic effects on bone tissue. Osteoblasts express insulin receptors, and activation of these receptors stimulates cell proliferation, collagen synthesis, alkaline phosphatase activity, and extracellular matrix production. Insulin also enhances osteoblast differentiation while reducing apoptosis, thereby promoting new bone formation. Experimental evidence further suggests that insulin signaling interacts with the IGF-1 (Insulin-like Growth Factor-1) pathway, amplifying osteogenic activity and accelerating fracture repair. Consequently, disturbances in glucose homeostasis—particularly those associated with diabetes mellitus—may significantly impair bone healing.

Hyperglycemia contributes to fracture-healing complications through multiple mechanisms. Persistent elevation of blood glucose promotes the formation of advanced glycation end products (AGEs), which accumulate within the collagen matrix, reducing its biomechanical properties and impairing normal bone remodeling. In addition, diabetes is associated with microvascular dysfunction, chronic inflammation, oxidative stress, impaired angiogenesis, and reduced osteoblast differentiation, all of which delay fracture repair. Conversely, severe hypoglycemia may also compromise tissue regeneration by limiting the energy supply required for proliferating cells during the inflammatory and reparative phases. Therefore, maintenance of physiological glucose homeostasis is essential throughout the healing process.

In addition to glucose metabolism, adequate availability of amino acids is indispensable for collagen synthesis, while calcium and phosphate are required for hydroxyapatite deposition and mineralization of newly formed bone. Vitamins, particularly vitamin D, vitamin C, and vitamin K, also play fundamental roles by regulating calcium homeostasis, collagen maturation, and bone matrix mineralization.

Several systemic hormones contribute to the metabolic regulation of fracture healing. Parathyroid hormone (PTH) stimulates bone remodeling and, when administered intermittently, enhances osteoblast activity and accelerates fracture healing. Growth hormone (GH) and IGF-1 promote cellular proliferation and matrix synthesis, whereas thyroid hormones regulate skeletal growth and remodeling. Conversely, prolonged exposure to glucocorticoids suppresses osteoblast differentiation, inhibits collagen synthesis, and increases bone resorption, thereby delaying fracture healing.

Nutritional status is another critical determinant of bone regeneration. Protein-energy malnutrition significantly reduces osteoblast activity, impairs collagen deposition, decreases angiogenesis, and compromises immune function, ultimately delaying fracture repair. Likewise, deficiencies in calcium, phosphorus, magnesium, zinc, copper, and essential vitamins adversely affect bone metabolism and increase the risk of delayed union or nonunion. Collectively, these findings demonstrate that successful fracture healing depends not only on adequate mechanical stability and local biological responses but also on systemic metabolic homeostasis. Optimization of nutritional status, endocrine function, and glycemic control should therefore be considered integral components of comprehensive fracture management.

As discussed by Spagliari *et al.* (2026), understanding the physiological mechanisms underlying insulin action and glucose metabolism provides important insights into the metabolic requirements of bone regeneration and highlights the critical role of systemic metabolic control in achieving optimal fracture healing outcomes.

FINALES CONSIDERATIONS

Fracture healing is a complex and highly coordinated biological process involving inflammatory, cellular, molecular, and metabolic mechanisms. Successful bone regeneration depends not only on adequate mechanical stability but also on angiogenesis, cellular activity, hormonal regulation, and proper nutritional status. A comprehensive understanding of these mechanisms is essential for optimizing fracture management and improving clinical outcomes.

REFERENCES

BUCHOLZ, Robert W.; COURT-BROWN, Charles M.; HECKMAN, James D.; TORNETTA III, Paul; MCQUEEN, Margaret M. **Rockwood & Green: fraturas em adultos**. 7. ed. Barueri: Manole, 2013.

HALL, John E.; HALL, Michael E. **Guyton & Hall: fundamentos de fisiologia.** 14. ed. Rio de Janeiro: Guanabara Koogan, 2023.

HANKENSON, Kurt D.; DISHOWITZ, Michael; GRAY, Christopher; SCHENKER, Matthew. **Angiogenesis in bone regeneration.** Injury, Amsterdam, v. 42, n. 6, p. 556–561, jun. 2011. DOI: 10.1016/j.injury.2011.03.035.

JUNQUEIRA, Luiz Carlos U.; CARNEIRO, José. **Histologia básica: texto e atlas.** 14. ed. Rio de Janeiro: Guanabara Koogan, 2023.

MARSELL, Richard; EINHORN, Thomas A. **The biology of fracture healing.** Injury, Amsterdam, v. 42, n. 6, p. 551–555, jun. 2011. DOI: 10.1016/j.injury.2011.03.031.

MULLIS, Brian H.; GASKI, Greg E. **Sinopse do tratamento do trauma ortopédico.** New York: Thieme Medical Publishers, 2020.

SHEEN, John R.; MABROUK, Ahmed; GARLA, Vijay V. **Fracture healing overview.** In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2026. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK551678/.

SKINNER, Harry B.; MCMAHON, Patrick J. **Ortopedia atual.** 5. ed. Porto Alegre: AMGH, 2015.

SPAGLIARI, Maria Emília; FERREIRA E SILVA, Eduarda Doffe; SILVA, Carlos Alberto da. **Eletrofisiologia do processo secretório da insulina: revisão.** In: Saúde e Medicina Contemporânea: avanços científicos, práticas clínicas e desafios globais. v. 2. São Paulo: Editora Científica Digital, 2026. p. 285–301. DOI: 10.37885/260622227.