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THE BIOLOGY OF FRACTURE HEALING

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Abstract

The biology of fracture healing is a complex biological process that follows specific regenerative patterns and involves changes in the expression of several thousand genes. Although there is still much to be learned to fully comprehend the pathways of bone regeneration, the over-all pathways of both the anatomical and biochemical events have been thoroughly investigated. These efforts have provided a general understanding of how fracture healing occurs. Following the initial trauma, bone heals by either direct intramembranous or indirect fracture healing, which consists of both intramembranous and endochondral bone formation. The most common pathway is indirect healing, since direct bone healing requires an anatomical reduction and rigidly stable conditions, commonly only obtained by open reduction and internal fixation. However, when such conditions are achieved, the direct healing cascade allows the bone structure to immediately regenerate anatomical lamellar bone and the Haversian systems without any remodeling steps necessary. In all other non-stable conditions, bone healing follows a specific biological pathway. It involves an acute inflammatory response including the production and release of several important molecules, and the recruitment of mesenchymal stem cells in order to generate a primary cartilaginous callus. This primary callus later undergoes revascularization and calcification, and is finally remodeled to fully restore a normal bone structure. In this article we summarize the basic biology of fracture healing.

Keywords

Bone healing; intramembranous; endochondral; callus; cartilaginous; periosteal; angiogenesis; revascularization; bone trauma; bone injury

INTRODUCTION

During the last two decades, our understanding of fracture healing has rapidly evolved. It is known that bone is one of few tissues that can heal without forming a fibrous scar. As such, the process of fracture healing recapitulates bone development and can be considered a form of tissue regeneration. However, despite the regenerative capacity of skeletal tissue, this

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biological process sometimes fails and fractures may heal in unfavorable anatomical positions, show a delay in healing or even develop pseudo-arthritis or non-unions.³²

Investigations in both humans and animal models have provided insight into the pathways that regulate the biologically optimized process of fracture healing and provide direction for further research to prevent its failure.¹⁶ The use of animal models have made it possible to investigate fracture healing from all perspectives such as histology, biochemistry and biomechanics and has hence been a very important tool in understanding fracture biology.⁶

To better understand new concepts and strategies to enhance the healing of fractures this review presents a basic summary of the current knowledge of the biology of fracture repair.

INDIRECT FRACTURE HEALING

Indirect (secondary) fracture healing is the most common form of fracture healing, and consists of both endochondral and intramembranous bone healing.¹⁷ It does not require anatomical reduction or rigidly stable conditions. On the contrary, it is enhanced by micro-motion and weight-bearing. However, too much motion and/or load is known to result in delayed healing or even non-union.²⁰ Indirect bone healing typically occurs in non-operative fracture treatment and in certain operative treatments in which some motion occurs at the fracture site such as intramedullary nailing, external fixation, or internal fixation of complicated comminuted fractures.^{35, 36}

The Acute Inflammatory Response

Immediately following the trauma, a hematoma is generated and consists of cells from both peripheral and intramedullary blood, as well as bone marrow cells. The injury initiates an inflammatory response which is necessary for the healing to progress. The response causes the hematoma to coagulate in between and around the fracture ends, and within the medulla forming a template for callus formation.¹⁸ Although it is known that inflammatory cytokines have a negative effect on bone, joints and implanted material when prolonged or chronic expression occur, a brief and highly regulated secretion of proinflammatory molecules following the acute injury is critical for tissue regeneration.¹⁸ The acute inflammatory response peaks within the first 24h and is complete after 7 days, although proinflammatory molecules also play an important role later in the regeneration as is described later in this article.¹²

The initial proinflammatory response involves secretion of tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6, IL-11 and IL-18.¹⁸ These factors recruit inflammatory cells and promote angiogenesis.⁴⁰ The TNF- α concentration has been shown to peak at 24h and to return to baseline within 72h post trauma.¹⁸ During this time-frame TNF- α is expressed by macrophages and other inflammatory cells, and it is believed to mediate an effect by inducing secondary inflammatory signals, and act as a chemotactic agent to recruit necessary cells.²⁸ TNF- α has also been shown *in vitro* to induce osteogenic differentiation of MSCs.¹¹ These effects are mediated by activation of the two receptors TNFR1 and TNFR2 which are expressed on both osteoblasts and osteoclasts. However, TNFR1 is always expressed in bone whereas TNFR2 is only expressed following injury, suggesting a more specific role in bone regeneration.^{3, 28} Among the different interleukins, IL-1 and IL-6 are believed to be most important for fracture healing. IL-1 expression overlaps with that of TNF- α with a biphasic mode. It is produced by macrophages in the acute phase of inflammation and induces production of IL-6 in osteoblasts, promotes the production of the primary cartilaginous callus, and also promotes angiogenesis at the injured site by activating either of its two receptors, IL-1RI or IL-1RII.^{28, 29, 40} IL-6 on the other hand, is only produced during

the acute phase and stimulates angiogenesis, vascular endothelial growth factor (VEGF) production, and the differentiation of osteoblasts and osteoclasts.⁴⁷

Recruitment of Mesenchymal Stem Cells (MSC)

In order for bone to regenerate, specific mesenchymal stem cells (MSCs) have to be recruited, proliferate and differentiate into osteogenic cells. Exactly where these cells come from is not fully understood. Although most data indicate that these MSCs are derived from surrounding soft tissues and bone marrow, recent data demonstrate that a systemic recruitment of circulating MSCs to the injured site might be of great importance for an optimal healing response.^{19, 27} Which molecular events mediate this recruitment is still under debate. It has long been suggested that BMP-2 has an important role in this recruitment, but data from our group indicates that this is not the case.² Indeed, BMP-2 is essential for bone repair⁴³ but other BMPs such as BMP-7 may play a more important role in the recruitment of progenitor cells.²

Current data suggest that stromal cell-derived factor-1 (SDF-1) and its G-protein-coupled receptor CXCR-4 form an axis (SDF-1/CXCR-4) that is a key regulator of recruiting and homing specific MSCs to the site of trauma.^{19, 27, 31} These reports show that SDF-1 expression is increased at the fracture site, and especially in the periosteum at the edges of the fracture. They also demonstrate that SDF-1 has a specific role in recruiting CXCR-4 expressing MSCs to the injured site during endochondral fracture healing.²⁷ The importance of this axis has been further verified as treatment with an anti-SDF-1 antagonist or genetic manipulation of SDF-1 and CXCR-4 impairs fracture healing. It has also been shown that transplanted MSCs only home to the fracture site if they express CXCR-4, whereas CXCR-4 negative MSCs do not have this ability.^{19, 27} Furthermore, recent data also demonstrate an important role for hypoxia inducible factor-1 α (HIF-1 α) in bone repair and its induction of VEGF in the revascularization process show that hypoxic gradients regulate MSC progenitor cell trafficking by HIF-1.^{9, 44}

Generation of a Cartilaginous and a Periosteal Bony Callus

Although indirect fracture healing consists of both intramembranous and endochondral ossification, the formation of a cartilaginous callus which later undergoes mineralization, resorption and is then replaced with bone is its key feature of this process. Following the formation of the primary hematoma, a fibrin-rich granulation tissue forms.³⁷ Within this tissue, endochondral formation occurs in between the fracture ends, and external to periosteal sites. These regions are also mechanically less stable and the cartilaginous tissue forms a soft callus which gives the fracture a stable structure.¹⁴ In animal models (rat, rabbit, mouse) the peak of soft callus formation occurs 7–9 days post trauma with a peak in both type II procollagen and proteoglycan core protein extracellular markers.¹⁵ At the same time, an intramembranous ossification response occurs subperiostally directly adjacent to the distal and proximal ends of the fracture, generating a hard callus. It is the final bridging of this central hard callus that ultimately provides the fracture with a semi-rigid structure which allows weight bearing.¹⁷

The generation of these callus tissues is dependent on the recruitment of MSCs from the surrounding soft tissues, cortex, periosteum, and bone marrow as well the systemic mobilization of stem cells into the peripheral blood from remote hematopoietic sites. Once recruited, a molecular cascade involves collagen-I and collagen-II matrix production and the participation of several peptide signaling molecules. In this process the transforming growth factor-beta (TGF- β) superfamily members have been shown to be of great importance. TGF- β 2, - β 3 and GDF-5 are involved in chondrogenesis and endochondral ossification, whereas BMP-5 and -6 have been suggested to induce cell proliferation in intramembranous

ossification at periosteal sites.^{12, 33} In addition, as noted above, BMP-2 has been shown to be crucial for initiation of the healing cascade, as mice with inactivating mutations in BMP-2 are not able to form callus in order to heal their fractures successfully.⁴³ Whether this is due to effects on mesenchymal stem cell proliferation and differentiation or effects on cell migration is still under debate.

Revascularization and Neoangiogenesis at the Fracture Site

Fracture healing requires a blood supply and revascularization is essential for successful bone repair.²⁵ In endochondral fracture healing, this not only involves angiogenic pathways, but also chondrocyte apoptosis and cartilaginous degradation as the removal of cells and extracellular matrices are necessary to allow blood vessel in-growth at the repair site.¹

Once this structural pattern is achieved, the vascularization process is mainly regulated by two molecular pathways, an angiopoietin-dependent pathway, and a vascular endothelial growth factor (VEGF)-dependent pathway.⁴² The angiopoietins, primarily angiopoietin-1 and 2, are vascular morphogenetic proteins. Their expression is induced early in the healing cascade, suggesting that they promote an initial vascular in-growth from existing vessels in the periosteum.³⁰ However, the VEGF pathway is considered to be the key regulator of vascular regeneration.²⁵ It has been shown that both osteoblasts and hypertrophic chondrocytes express high levels of VEGF, thereby promoting the invasion of blood vessels and transforming the avascular cartilaginous matrix into a vascularized osseous tissue.²⁵ VEGF promotes both vasculogenesis, i.e. aggregation and proliferation of endothelial mesenchymal stem cells into a vascular plexus, and angiogenesis, i.e. growth of new vessels from already existing ones.²⁴ Hence, VEGF plays a crucial role in the neoangiogenesis and revascularization at the fracture site. Its importance in these processes is further supported by the observations that addition of excessive VEGF promotes fracture healing, whereas blocking of VEGF-receptors inhibit vascular in-growth and delays or disrupts the regenerative process.^{1, 25} Several other factors may also be involved in these responses, having pro-angiogenic effects, such as the synergistic interactions of the BMPs with VEGF and the role of mechanical stimuli to enhance angiogenic activities in a VEGFR2-dependent manner.^{1, 24}

Mineralization and Resorption of the Cartilaginous Callus

In order for bone regeneration to progress, the primary soft cartilaginous callus needs to be resorbed and replaced by a hard bony callus. This step of fracture healing, to some extent, recapitulates embryological bone development with a combination of cellular proliferation and differentiation, increasing cellular volume and increasing matrix deposition.⁷ The connection between bone regeneration and bone development has been further strengthened by a recent understanding of the role of the Wnt-family of molecules, which is of great importance in embryology and has also been shown to have an important role in bone healing. The Wnt-family is thought to regulate the differentiation of pluripotent MSCs into the osteoblastic lineage and, at later stages of development, to positively regulate osteoblastic bone formation.¹⁰

As fracture callus chondrocytes proliferate, they become hypertrophic and the extracellular matrix becomes calcified. A cascade orchestrated primarily by macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG) and TNF- α initiates the resorption of this mineralized cartilage.^{4, 18} During this process M-CSF, RANKL and OPG are also thought to help recruit bone cells and osteoclasts to form woven bone. TNF- α further promotes the recruitment MSC with osteogenic potential but its most important role may be to initiate chondrocyte apoptosis.¹⁸ The calcification mechanism involves the role of mitochondria, which accumulate calcium-

containing granules created in the hypoxic fracture environment. After elaboration into the cytoplasm of fracture callus chondrocytes, calcium granules are transported into the extracellular matrix where they precipitate with phosphate and form initial mineral deposits. These deposits of calcium and phosphate become the nidus for homogeneous nucleation and the formation of apatite crystals.²⁶ The peak of the hard callus formation is usually reached by day 14 in animal models as defined by histomorphometry of mineralized tissue, but also by the measurement of extracellular matrix markers such as type I procollagen, osteocalcin, alkaline phosphatase and osteonectin.¹⁵ As the hard callus formation progresses and the calcified cartilage is replaced with woven bone, the callus becomes more solid and mechanically rigid.¹⁷

Bone Remodeling

Although the hard callus is a rigid structure providing biomechanical stability, it does not fully restore the biomechanical properties of normal bone. In order to achieve this, the fracture healing cascade initiates a second resorptive phase, this time to remodel the hard callus into a lamellar bone structure with a central medullary cavity.¹⁸ This phase is biochemically orchestrated by IL-1 and TNF- α , which show high expression levels during this stage, as opposed to most members of the TGF- β family which have diminished in expression by this time.^{1, 34} However, some BMPs such as BMP2, are seemingly also involved in this phase with reasonably high expression levels.³³

The remodelling process is carried out by a balance of hard callus resorption by osteoclasts, and lamellar bone deposition by osteoblasts. Although the process is initiated as early as 3–4 weeks in animal and human models, the remodelling may take years to be completed to achieve a fully regenerated bone structure.⁴⁵ The process may occur faster in animals and younger patients. Bone remodelling has been shown to be a result of production of electrical polarity created when pressure is applied in a crystalline environment.⁵ This is achieved when axial loading of long bones occur, creating one electropositive convex surface, and one electronegative concave surface, activating osteoclastic and osteoblastic activity respectively. By these actions the external callus is gradually replaced by a lamellar bone structure, whereas the internal callus remodelling re-establishes a medullary cavity characteristic of a diaphyseal bone.⁵

For bone remodelling to be successful, an adequate blood supply and a gradual increase in mechanical stability is crucial.⁸ This is clearly demonstrated in cases where neither is achieved, resulting in the development of an atrophic fibrous non-union. However, in cases in which there is good vascularity but unstable fixation, the healing process progresses to form a cartilaginous callus, but results in a hypertrophic non-union or a pseudoarthrosis.²⁰

DIRECT FRACTURE HEALING

Direct healing does not commonly occur in the natural process of fracture healing. This since it requires a correct anatomical reduction of the fracture ends, without any gap formation, and a stable fixation. However, this type of healing is often the primary goal to achieve after open reduction and internal fixation surgery. When these requirements are achieved, direct bone healing can occur by direct remodeling of lamellar bone, the Haversian canals and blood vessels. Depending on the species, it usually takes from a few months to a few years, before complete healing is achieved.³⁷

Contact Healing

Primary healing of fractures can either occur through contact healing or gap healing. Both processes involve an attempt to directly re-establish an anatomically correct and biomechanically competent lamellar bone structure. Direct bone healing can only occur

when an anatomic restoration of the fracture fragments is achieved and rigid fixation is provided resulting in a substantial decrease in interfragmentary strain. Bone on one side of the cortex must unite with bone on the other side of the cortex to re-establish mechanical continuity. If the gap between bone ends is less than 0.01 mm and interfragmentary strain is less than 2%, the fracture unite by so-called *contact healing*.⁴¹ Under these conditions, cutting cones are formed at the ends of the osteons closest to the fracture site.²² The tips of the cutting cones consist of osteoclasts which cross the fracture line, generating longitudinal cavities at a rate of 50–100 $\mu\text{m}/\text{day}$. These cavities are later filled by bone produced by osteoblasts residing at the rear of the cutting cone. This results in the simultaneous generation of a bony union and the restoration of Haversian systems formed in an axial direction.^{23, 37} The re-established Haversian systems allow for penetration of blood vessels carrying osteoblastic precursors.^{15, 21} The bridging osteons later mature by direct remodelling into lamellar bone resulting in fracture healing without the formation of periosteal callus.

Gap Healing

Gap healing differs from contact healing in that bony union and Haversian remodelling do not occur simultaneously. It occurs if stable conditions and an anatomical reduction are achieved, although the gap must be less than 800 μm to 1 mm.²³ In this process the fracture site is primarily filled by lamellar bone oriented perpendicular to the long axis, requiring a secondary osteonal reconstruction unlike the process of contact healing.³⁹ The primary bone structure is then gradually replaced by longitudinal revascularized osteons carrying osteoprogenitor cells which differentiate into osteoblasts and produce lamellar bone on each surface of the gap.⁴¹ This lamellar bone, however, is laid down perpendicular to the long axis and is mechanically weak. This initial process takes approximately 3 and 8 weeks, after which a secondary remodelling resembling the contact healing cascade with cutting cones takes place. Although not as extensive as endochondral remodelling, this phase is necessary in order to fully restore the anatomical and biomechanical properties of the bone.⁴¹

CONCLUSION

There are several pathways through which bone can heal, yet the unique attribute of bony repair is that it occurs without the development of a fibrous scar. This designates the process of fracture healing as a form of tissue regeneration. In order to achieve a complete regeneration of a fully functional bone, many interrelated anatomical, biomechanical and biochemical processes must occur in a well orchestrated manner.

While this article describes the essential components of the process of fracture healing, other mechanisms have also been shown to have important roles in bone regeneration: such as the actions of metalloproteinases, the involvement of several endocrine systems affecting phosphate and calcium homeostasis, and the hematopoietic system and its regulation of the mesenchymal stem cell progenitors that are crucial for bone and vascular regeneration.^{13, 38, 46}

Although currently available data provide a detailed picture of the complex biological pathways through which bone is regenerated, much remains to be understood and many questions remain. It is anticipated that with the development of new imaging technologies and advanced systems for molecular analysis many of these questions will be answered.

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