Play and players in bone fracture healing match

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Summary

Bone fractured healing is a specialized wound-healing response that leads to regeneration without scar restoring its own ability of mechanical loading. The four stage classification of fracture healing process, by John Hunter, is still the frame in which the new biological and molecular findings settle in. Nowadays the fracture healing is pictured like a playground where growth and differentiation factors, hormones, cytokines, and extracellular matrix play with bone and cartilage forming primary cells and muscle mesenchymal cells in a well orchestrated series of biological events. The ongoing knowledge of cellular and molecular interactions between blood vessels and bone cells shows great promise to enhance fracture management and the unsuccessfull process of bone healing.

KEY WORDS: fracture healing, callus, molecular osteogenesis, stem cell, growth factor.

Introduction

Fracture healing is a unique physiologic process in which bone repairs itself with the help of surrounding tissues, as periosteum, blood, bone marrow, external soft tissue, and restores its own ability of mechanical loading. This process involves a chain of cellular and molecular events, many of which are similar to those occur in soft tissue wound healing. The essential difference between these two healing processes is the absence of the scar at the healing end instead of its presence in the other one. If successful, bone regenerates itself with newly formed bone remodelling into the original anatomy in children and in a mechanically stable lamellar structure in adults (1). There are cellular and biochemical parallel pathways between the fracture healing with callus and the growth plate during development, whereas the first one process occur on a temporal rather than a spatial frame (2).

Nowadays the fracture healing is pictured like a playground where growth and differentiation factors, hormones, cytokines, and extracellular matrix play with bone and cartilage forming primary cells and muscle mesenchymal cells in a well orchestrated series of biological events.
Understanding this complex process may enable us to work up new strategies to enhance fracture management and the unsuccessfull process of bone healing.

Brief notes on bone healing history

Since 1700s the basic biology of fractured bone healing has been detected and the four-stage classification of bone repair, consisting of inflammation, soft callus, hard callus and remodelling (3), credited to John Hunter (1728-1793). The key point of the following debate was to understand which the players of bone healing process were and many were the working hypotheses. John Hunter agreed with the theory of Albrecht Haller (1708-1777) that bone was deposited from the vascular network around the injured zone (4). Contrary to this, H.L. Duhamel stated that bone was formed from “cambium layer”, the osteogenic side of periosteum (5). By another side, first John Belchier and after John Goodsir (1814-1867) focused their attention on osteoblast as the main bone-builder cells in fracture healing process (6). In according to them, W. MacEwen (1848-1924) assessed the not relevant role of the surgical preservation of the periosteum layer.
At the same time Louis Xavier Ollier (1830-1900) supported that bone healing was due to periosteum, bone marrow and bone, and periosteum tissue had to be preserved in surgical approaches of bone fractures.
Following this school of thought based on the surgical preservation of the injured zone, two new surgical methodologies were developed in 1930s by Raoul Hoffman (1881-1972) with the concept of closed reduction of fractures and its fixation with external devices (7) and by Gerhardt Kuntscher (1900-1972) with his nonreamed intramedullary nailing, a vascular-sparing technique that preserve the fracture periosteal environment (8).
In 1990s there were another two cornerstones in the understanding of bone healing process. The first one was the studies of Robert Danis (1880-1962) who discovered that interfragmentary compression and compression plating resulted in different type of bone healing, named primary (direct) bone healing, without a relevant presence of callus. This new school of fracture healing thought focused on the bone tissue management was promulgated by the A.O. Group, founded by Willenegger, Mueller, Allgoewer and Schneider. Their school introduced a standardised surgical treatment for fractures in the early 1950s and it is of great significance nowadays (9). By that time, the callus process of fracture healing was termed secondary (indirect) bone healing.
In accord with the callus healing process and diametrically opposed to the first one, the second cornerstone was the innovative theory of Gavriil A. Ilizarov (1921-1992), called distractive osteogenesis and based on the biological principle of tension stress. According to this principle, gradual controlled distraction of the bone ends not only stimulates bone production but also

supports the regeneration of the overlying tissue. This was realized with a no rigid external fixation and temporal sequential distraction (10).
The contributions of all authors, those mentioned and the others not, gave the basics on which the present view of bone fractured healing is improving, as a spatial and temporal coordinated action of several different cell types, proteins and the expression of hundreds of genes (11).

Molecular osteogenesis

The modern regard of fracture healing process account it as a molecular and genetic chain in which all the rings are controlled by balanced molecular systems that regulate the cellular activations.
The promoting molecules of the fracture healing process can be divided into three main groups: 1) the pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) that are expressed first in the inflammatory phase and later in the remodelling phase (12, 13); 2) the growth and differentiation factors, including transforming growth factor-β superfamily (GDFs, BMPs, TGF-β) platelet-derived growth factor (PGDF), fibroblast growth factor (FGF) and insulin-like growth factor (IGF), that are operative few hours after the fracture time during all the reparative phase (14, 15); 3) the metalloproteinases and angiogenic factors, including vascular-endothelial growth factors (VEGF) and angiopoietin 1 and 2, that start in the second part of the endochondral ossification for metalloproteinases and VEGF, and go on up to the conclusion of the remodelling phase providing an adequate blood flow (16).
Numerous inhibitory molecules, regulating different signalling pathways, are discovered in the last years and their activities occur not only in bone repairing but also in embryonic bone tissue development and in adult bone tissue maintenance. Various are the levels at witch inhibition occurs as extracellular, intracellular, receptor or nucleus sites, demonstrating the complexity of the physiological molecular processes. The antagonist molecules of BMPs, the proteins with the greatest osteoinductive properties, are divided according to the action's level: 1) extracellular level as noggin, chordin, sclerostin, follistatin, BMP3 and Ahsg (a2-Hs-glycoprotein); 2) receptor level as a pseudoreceptor called BAMBI (BMP and activin membrane bound inhibitor) observed during embryogenic development, with a unclear activity in damaged adult tissues; 3) intracellular level as inhibitory SMADs. The results on inhibitory role of these molecules come from embryonic or in vitro studies and further research is needed to assess their actions in adult tissue (16, 17).

The ways of bone fracture healing

The main concept to understand the chain of cellular and molecular events that leads to bone fracture healing is to have a comprehensive view of the spatial and temporal connections of the different repairing events in fractured bone.
There are four components to the injury site; the cortex, the periosteum, the bone marrow, and the external soft tissues, all of which contribute to the healing process. The extent to which each component is involved depends on the conditions present at the injured tissue, such as the level of growth factors, hormones, nutrients, pH, oxygen tension, the electrical environment, and the mechanical stability of the fracture (20).
As seen previously, there are two known ways by that the bone heals after a broken trauma. This two branches of fracture healing, direct or primary and indirect or secondary, is based on the histological evidences that occur during the repair process. Worldwide the majority of fractures, treated or not, with some no rigid fixation, repair by indirect way. Ongoing closer review of the surgical techniques, which lead or not to successful reparative osteogenesis, can now suggest a synthesis of these ways as parts of a unique process.

Direct cortical fracture healing

Direct (primary) cortical fracture healing is a process aimed at restoring mechanical continuity involving the attempt to bridge the fracture gap by the rebuilding of a new Haversian cortical system. It occurs when the fracture fragments are reduced anatomically and fixed rigidly (18). Under these conditions, osteoclasts resorb the dead bone on the fracture ends starting a tunnelling resorptive processes and breaking new ground to the remodelling units known as "cutting cones" (19). This network of vascular endothelial cells and perivascular mesenchymal cells produce new vessels and provide the osteoprogenitor cells that differentiate into osteoblasts in order to produce new osteonal bone. Because little or no callus formation is noted, cortex tissue is recognized as the main player of this process with a little participation from the periosteum, external soft tissues, or bone marrow (20). Nowadays there is a critical review of the carrying out of primary cortical fracture healing and some authors agree to consider this process not a healing in the strict sense of the word but a side effect of internal removal of necrotic bone that goes to a bone remodelling in a longer time rather than callus healing. It may be asserted that absolute stability of surgical fixation directly leads the fracture to the remodelling phase of physiological fracture healing process skipping the reparative one and taking place of the callus stabilizing role.

Indirect fracture healing

Following the Hunter's frame above mentioned, indirect (secondary) fracture healing process is divided in three main groups: the inflammatory phase, the reparative phase and the remodelling phase.
Each of them have a different steps even if no consecutive temporal and spatial sequence can be pictured because one or several of these occur simultaneously or in a partial overlap. It depends on the fracture type and location, the chosen technique for treatment, the regional vascular network and the activated molecular system of promoting/inhibiting fracture repair.

The *inflammatory phase* is a trauma response shared by soft tissue wound healing too that includes three different stages:
1. the *haematoma stage* in which, following injury, the vascular disruption leads to acute necrosis, local acidosis and hypoxia of bone, periosteum, bone marrow and soft tissues. The following activation of thrombotic factors brings to the blood clot formation in fracture site. Hematoma is the source of signalling molecules that have the capacity to initiate the cascades of cellular events that are critical to fracture healing (22). Osteocytes deprived of nutrition at the fracture ends die and play a passive role in the repair process;
2. the *inflammatory stage* is a reaction similar to that of soft tissue injuries. Macrophages and other immune cells as monocytes, lymphocytes and leucocytes, are recruited to the fracture site and are essential in secreting proinflammatory cytokines (23). Their main effect are: induction of extracellular matrix synthesis, angiogenesis stimulation, chemotactict effect on circulating immune and mesenchimal cells, recruitment of endogenous fibroblasts (24). Activated platelets release growth and differentiation factors to the induction of ossification.

3. the *granulation tissue stage* is the following organisation of haematoma; there is growth of capillary buds from endosteal circulation (Rhinelander, 1974), a process of cleaning out the damaged material, resorbtion of dead calcified bone, macrophage activation and modulation of undifferentiated mesenchymal cells into fibroblasts, chondroblasts and osteoblasts. The transient granulation tissue is replaced by fibrocartilage.

Microenvironment is acidic in inflammatory phase; it moves towards neutrality during reparative phase and becomes alkaline in the remodelling one (20). It may be correlate to the progressive increase of the O2 tension in the fracture site during the healing process.

The **reparative phase** is a unique process to bone, begins within several days of the initial inflammatory response and it is generally enhanced by micromotion and inhibited by rigid fixation (19). This specialized sequence involves committed osteoprogenitor and undifferentiated mesenchymal cells into the callus tissue that will further differentiate into skeletal cells. Two concomitant but separate bone forming pathways are initiated to heal the fracture:

1. the *intramembranous ossification stage* forms bone directly without the development of an intermediate cartilage tissue. From a histological point of view, the resulting callus is described as hard callus. This pathway starts at three sites: the first two are the periosteum and the endosteum farther from the fracture site (20) where undifferentiated mesenchymal cells and committed osteoprogenitor cells are induced to differentiate directly into osteoblasts (25); the third site is the bone marrow where, in the early phase, endothelial cells transform themselves expressing an osteoblastic phenotype (26). The presence of a well vascular network that guarantees a high O_2 tension is the necessary condition that allows the performing of intramembranous ossification (27).
2. the *endochondral ossification stage* is a double process that develops adjacent to the fracture site. The first process is the chondrogenesis that involves the recruitment, attachment, and proliferation of mesenchymal cells that differentiate into growth cartilage while angiogenesis develops. These cells initially synthesize an extracellular matrix composed predominantly of type II collagen and proteoglycans (28). Cartilage forms particularly in regions of low O_2 tension and is replaced with woven bone when the angiogenetic process develops and elevates O_2 level (29). From a histological point of view, the resulting callus is described as soft callus. The second process, partially overlapping with the first one, is the endochondral ossification, a sequence of cartilage calcification, cartilage removal and bone formation. In this sequence chondrocytes release protease and phosphatase enzymes in order to degrade the proteoglycans and to provide phosphatase ions that precipitate in the extracellular matrix with calcium delivered from mitochondria of hypertrophic chondrocytes. When chondrocytes undergo apoptosis, chondroclasts activate and resorb the mineralized cartilage matrix sending a signal to enable vascularisation of this tissue bringing perivascular mesenchymal stem cells. The following differentiation into osteoprogenitor cells and then into bone-forming osteoblasts provides the replacement of calcificated matrix with woven bone, oriented along the neovascular pathway (30).

The **remodelling phase** is composed of two overlapping processes in order to replace the newly formed bone with definitive bone tissue (lamellar bone) and to reduce the callus size restoring a normal vascular supply respectively. In this last phase strength of bone returns almost like in normal bone and chance of re-fracture decreases (19, 31).

In the *first process* the basic multicellular unit (BMU) is the main actor that drives the bone replacement following a stereotyped pattern as activation-resorption-bone apposition. Several types of cells are involved as osteoclasts developed from hematopoietic progenitors, osteoblasts derived from mesenchymal stem cells, capillaries and extracellular matrix linked each other by the molecular signalling derived from circulating hormones, cytokines and growth factors. BMU substitutes the woven bone with the lamellar one and replaces callus between the ends of compact bone with new osteons aligning themselves parallel to the compression and tension stress and strain caused by mechanical use and muscle forces. Every BMU works 1 to 4 years to replace callus with functionally competent lamellar bone (32).
In the *second process* osteoclasts act to remodel the external surface of bone and decrease the size of the callus restoring a normal anatomy in the youngs only. Endosteal callus is reabsorbed slowly until the original shape of the cortex is restored.
It takes place in periosteum and endosteum layers simultaneously with the first process and goes on from 1 to 4 years (33).

Conclusion

The four-stage classification of bone repair, credited to John Hunter by 1768, is still the current model in which the fracture healing research sets the new biological and molecular findings. The ongoing understanding of physiological fracture healing process shows a complex frame that involves genetical, biological and mechanical sides, but numerous aspects have to be still clarified. Therefore the actual knowledge is drawing new osteogenetical pathways that drive to mimic the natural bone healing process in case of impaired repairing. More than in the past, osteogenesis and angiogenesis seem to be the main linking processes that support not only the bone regeneration but also its maintenance and development.

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