Play and players in bone fracture healing match

Lorenzo Marzona, MD
Bernardo Pavolini, MD

U.O. di Ortopedia e Traumatologia
AUSL1 Massa e Carrara
Regione Toscana - Italy

Address for correspondence:
Lorenzo Marzona, MD
U.O. di Ortopedia e Traumatologia
Presidio Ospedaliero SS Giacomo e Cristoforo
Via Sottomonte 1, 54100 Massa, Italy
Ph. +39 0585 493801
Fax +39 0585 493440
E-mail: lorenzomarz@tiscali.it

This paper has been written entirely by the authors, and has received no external founding. The authors have non significant financial interest or other relationship

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 unsuccessful 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 unsuccessful 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 Goodris (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 periosteal 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 interfragmen- tary compression and compression plating resulted in differ- ent 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 Wil- lenegger, Mueller, Allgower and Schneider. Their school intro- duced 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 sec- ondary (indirect) bone healing.

In accord with the callus healing process and diametrically opposed to the first one, the second cornerstone was the innova- tive 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 re-
alized with a no rigid external fixation and temporal sequential
distract (10).

The contributions of all authors, those mentioned and the oth-
ers not, gave the basics on which the present view of bone
fractured healing is improving, as a spatial and temporal coor-
dinated 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 con-
trolled 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 cy-
tokines, 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 trans-
growth factor-β (TGF-β) superfamily (GDFs, BMPs, TGF-β); platelet-derived growth factor (PDGF), 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, in-
cluding vascular-endothelial growth factors (VEGF) and an-
giopoietin 1 and 2, that start in the second part of the endo-
chondral 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 tis-
se development and in adult bone tissue maintenance. Vari-
ous are the levels at which inhibition occurs as extracellular, in-
tracellular, receptor or nucleus sites, demonstrating the com-
plicity of the physiological molecular processes. The antagonist
molecules of BMPs, the proteins with the greatest osteoinduc-
tive properties, are divided according to the action’s level: 1) ex-
tracellular level as noggin, chordin, sclerosin, follistatin, BMP3
and Ahs (a2-Hs-glycoprotein); 2) receptor level as a pseudore-
derceptor called BAMBI (BMP and activin membrane bound in-
hibitor) observed during embryonic development, with a un-
clear activity in damaged adult tissues; 3) intracellular level as
inhibitory SMADs. The results on inhibitory role of these mole-
cules come from embryonic or in vitro studies and further re-
search 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 mole-
cular 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 pe-
rioneum, 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, hor-
mones, nutrients, pH, oxygen tension, the electrical environ-
ment, 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 synthe-
sis 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 anat-
omically and fixed rigidly (18). Under these conditions, os-
teoclasts resorb the dead bone on the fracture ends starting a
tunneling 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 tis-
ue 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 fix-
dation 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 (sec-
dondary) 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 tech-
nique for treatment, the regional vascular network and the acti-
vated 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 hypox-
ia 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 heal-
ing (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 tis-
sue injuries. Macrophages and other immune cells as mono-
cytes, lymphocytes and leucocytes, are recruited to the frac-
ture site and are essential in secreting proinflammatory cy-
tokines (23). Their main effect are: induction of extracellular
matrix synthesis, angiogenesis stimulation, chemotactict ef-
cect on circulating immune and mesenchymal cells, recruit-
ment of endogenous fibroblasts (24). Activated platelets re-
lease 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, resorption 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 progress 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 cal- 
lus 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 cal- 

lus 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 O2 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, 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 hypotrophic chondrocytes. When chondrocytes undergo apoptosis, chondroclasts activate and resorb the 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 1766, 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 generational, 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.

References

2. Belchier J. Account of the bones of animals being made red by alli-
ent conditions of bone fragment fixation (experimental study). Ek-
4. Duhamel HL. Sur le developpement et la crue des os des animaux. Histoire et Memoires De l'Academie Des Inscriptons et Belles Let-
tres. 1742;2:481.
5. Belchier J. Account of the bones of animals being made red by alli-
ent only. Phil Trans London. 1736;42:287.
11. Schwartz RP. The historical herivation of the physiological princi-
15. Veillette CJH, McKee MD. Growth factors – BMPs, DBMs, and buffy coat products; are there any proven differences amongst them? Injury, Int J Care Injured. 2007;38(S1):S38-S48.