Jona J. Sela · Itai A. Bab *Editors*

Principles of Bone Regeneration

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Preface

Bone healing is the process whereby deficiencies and discontinuities in bone tissue are repaired by a regeneration process that rescues the biomechanical properties of the skeleton. Inevitably, this process involves an ultimate net gain in the amount of mineralized matrix at the affected sites. This gain may progress slowly, as in the case of the positive shift of bone remodeling balance induced in the osteoporotic skeleton by bone anabolic agents, or, as an outburst of bone formation and remodeling characteristic of the bone tissue reaction to traumatic insults. The importance of bone healing to medicine and biomedical research is illustrated by the number of publications on the different aspects of the subject, which exceeded 2,000 in 2011 alone.

Either form of bone healing is affected by a multitude of genetic, environmental, mechanical, cellular, and endocrine variables which eventually lead to changes in gene expression that enhance the guided action of osteoblasts (and chondroblasts) to lay down bone that restores, or even improves, the skeletal load bearing capacity and body motion. Needless to say, osteoclasts are also involved in shaping the healed tissue. Recent breakthroughs in understanding the regulatory aspects of bone formation and resorption, at the basic, translational, and clinical arenas, offer new modalities to induce, enhance, and guide repair processes in bone for the benefit of millions of patients with conditions such as osteoporosis, nonunion fractures, critical size defects, orthodontic tooth movement, periodontal bone loss, intraosseous implants, and deformed bones.

An immense number of approaches to treating these conditions are currently under basic, preclinical, and clinical investigations. They range from the development of sophisticated biomaterials for implant surgery, identification of neurotransmitters active in bone and other molecular drug targets, new drugs engineered by cutting edge pharmacological and molecular approaches, and advanced methods for tissue engineering and gene and cell therapies.

Because of the multidisciplinary nature of these efforts, this book addresses the modern aspects of bone healing, with a special attempt to enhance the convergence of the different experimental and clinical approaches designed for the study and treatment of bone healing in its diverse forms and under varying conditions. The information and ideas provided should have value not only for the experimental skeletal biologist and clinician treating bone conditions but also for a general interpretation of healing and regenerative processes in mammals.

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Born 1939, Jerusalem; D.M.D. 1966, Hebrew University

Appointments at the Hebrew University: Lecturer 1970; Senior Lecturer 1974; Associate Professor 1977; Professor 1981

Director, Division of Oral Pathology, 1989–2002

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Honorary Research Fellow, Hard Tissue Unit, Department of Anatomy, University College London, UK, 1977

Visiting Scientist, Department of Human Genetics, UCL, UK, 1983

Lady Davis Visiting Professor, Department of Morphological Sciences, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, 1986–1987

Guest Professor Medical Center Steglitz, Free University of Berlin, 1987

Chief-Superintendent, Head, Forensic Odontology, Israel Nat. Police 1987–1996; Chairman, Terminology Committees on Biological Sciences and Dental Medicine, The Hebrew Language Academy 1985–date

Visiting Professor UKBF, Free University of Berlin, Germany, 1996

Membership, Fellowship and Chairman

Chairman, Israel Society of Electron Microscopy, 1988–1993

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Research Interest and Projects

Gene-expression of bone cells around orthopedic implants. Automated image analysis supported by computerized quantitative morphometry for the study of observations obtained by electron and light microscopy in normal and pathological conditions. Development of novel computerized quantitative histomorphometric methodology to study oral and systemic pathological changes in cancer and wound healing.

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Research Interests and Projects

The bone laboratory is engaged in multidisciplinary research studying the mechanisms involved in skeletal remodeling, metabolic bone diseases, and the integration of endosseous implants. The laboratory studies the effects of different hormone and growth factor derived drugs on bone remodeling, bone mass, and healing of bone injuries. Recently, the laboratory has been engaged in the development of a new scientific field, neuropsychoosteology, which explores the bidirectional interaction between the brain and the skeleton. The methodological approaches employed in the laboratory encompass micro-computed tomography and histomorphometry, cellular and molecular biology, genetics, biochemistry and medicinal chemistry.

Endocannabinoids: Metabolites of Phospholipids as Modulators of Cell Function. Funding: German Ministry of Science/SFB 645. Central IL-1 Receptor Signaling and Bone Mass. Funding: German-Israeli Foundation. Bone Anabolic Agents. Funding: National Institute of Health, USA. Cannabinoid Therapy for Osteoporosis. Funding: commercial sources. Oleoyl Serine and Bone Mass. Funding: US–Israel Binational Science Foundation. Depression and Bone Loss. Israel Science Foundation. Effect of Cannabinoids on Repair Processes in Bone. Funding: Israel Anti-drug Authority. Cannabinoids and Brain Function. Funding: National Institute of Health, USA.

Contents

Chapter 1
Healing of Bone Fracture: General Concepts

Jona J. Sela and Itai A. Bab

The skeleton is frequently exposed to accidental and iatrogenic insults. Bone, similar to several other tissues, portrays a marked potential for regeneration and repair. Generally, healing proceeds until a complete restoration of the osseous function and anatomy is achieved. Cellular and molecular participants are similar in healing processes of bone and other tissues of mesenchymal origin. Skeletal injury initiates a multifaceted healing process since additional non-osseous tissues are involved. In view of potential complications in the healing process, a methodological approach to expected cellular and molecular therapeutic targets is required. The study of such targets in skeletal morphogenesis reveals that the phases of bone healing display striking similarities to osseous growth and development [1–5].

Classification of the patterns of bone healing is based on a variety of events and factors that influence injury and repair. Currently, the extent of tissue loss is considered to be of critical significance. It is clear that the increase in the amount of bone loss is in direct correlation with the delay in healing. Therefore, the extent of the discontinuity between the fractured edges is accepted to serve as streamline factor for the sorting of the different types of healing. Consequently, the following two major patterns of bone repair are defined:

(a) Healing following close approximation and rigid compression of the fractured edges. This could be considered as healing in primary intention with a minimal replacement of the injured bone by intermediary tissues. The process is concluded

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by a complete union between the fractured edges. Bone healing in this situation is described to occur in both lamellar and trabecular bones in instances of tight proximity of less then 0.1 mm between fractured edges with rigid stabilization. The suggested theory is that this type of healing is mediated by periosteal and endosteal tissues of the intraosseous Haversian system, marrow-derived vessels and mesenchymal cells, osteoblasts, and osteoclasts. Regeneration is characterized by bone remodeling parallel to the streamline of the osteon system. This union is formed by continuous ossification in first intention without cartilaginous or woven bone formation. The osteoclasts, engaged in necrotic bone resorption, are accompanied by the osteoblasts that form lamellar bone. Remodeling of the repaired bone is minimal in this environment consisting of minimal interfragmentary space [4]. *The concept of direct continuous bone regeneration is controversial.* It lacks basic scientific support with histological evidence in the literature. Most researchers would dispute the idea that healing could occur without formation of transient tissues between the fractured edges. It should be noted that a minimal hemorrhage is evident in all cases of trauma, and hence a blood clot, even if minimal, would develop in the fracture area serving as initial matrix for the proliferation of the involved cellular population. However, the theory on direct bone repair serves as a "scientific" justification for various orthopedic procedures. In these instances, the fracture edges are tightly pressed together. Clinical articles report a high rate of successful complete union [4]. It could be pointed out that "green stick" and "stress" fractures would probably heal in a similar manner.

(b) Healing with separated fracture edges involving intermediary tissues. These fractures are characterized by a significant gap formed between the edges with an extent of less than the diameter of the bone. Cases of such discontinuity are proven to heal regularly with artificial fixation. This type of bone healing is probably the most abundant one and is defined as healing in secondary intention (Fig. 1.1).

Clinically, fracture repair is optimized without a tight approximation of the severed edges. The course of healing constitutes several processes along the following possible stages: blood clotting, inflammatory response, granulation tissue formation, macrophage and osteoclast activity, significant bone resorption; formation of cartilaginous callus (endochondral repair) with calcification and young osseous matrix of primary bone. The continuance of the process is characterized by mineralization of the matrix.

It should be pointed out that the newly formed calcifying tissue can serve as a stabilizing but not as a weight-bearing component. Woven bone and cartilage serve as bridging templates. Complete maturation is accomplished by bone remodeling to form biomechanically compatible structures. Osseous regeneration is dependent upon several clinical issues such as location, extent of tissue loss, fracture mobility, infection, and types of reconstructive materials and systemic conditions. In addition, bone regeneration is usually accompanied by restoration of the collaterally damaged tissues, i.e., joints, cartilage, muscles, tendons, ligaments, skin, mucous membranes, bone marrow, periodontal ligament, etc. [3–5].

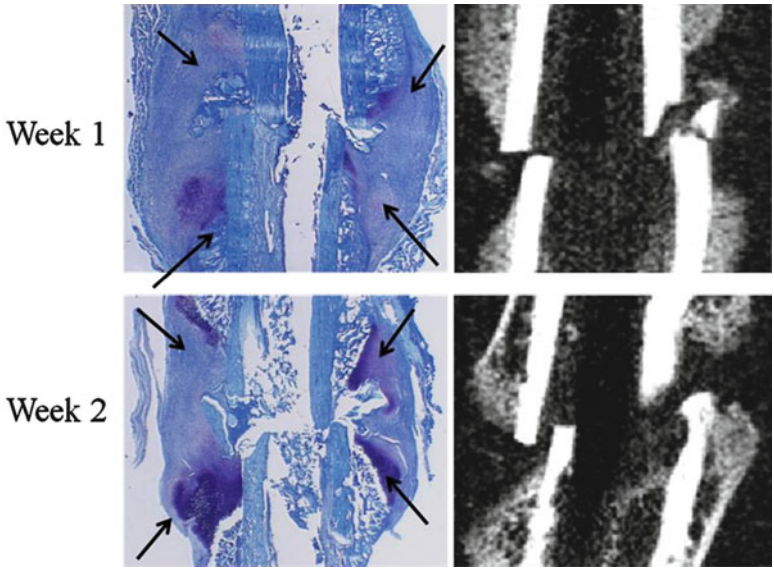

Fig. 1.1 Long bone fractures and callus in first and second weeks of healing. Note, Pairs of histological and μCT representations. *Week 1*: Callus is constructed large cartilaginous component (*arrows*), initially calcified. *Week 2*: Higher calcification (*intense violet*) and reduction of callus size

The natures of the genetic and molecular triggers that initiate and regulate the signaling pathways in the process of cellular activation in bone healing are starting to be disclosed [5–8].

Following trauma, molecules participating in fracture healing comprise pro-inflammatory cytokines, i.e., interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) that are expressed first in the inflammatory phase and later in the remodeling phase. This stage is followed by the involvement of 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 [8, 9]. Subsequently, endochondral ossification is characterized by the activities of metalloproteinases, vascular endothelial growth factors (VEGF), and angiopoietin 1 and 2. Molecules antagonist to bone morphogenetic proteins (BMPs) have been identified. Noggin, chordin, sclerostin, follistatin at extracellular setting and BAMBI (BMP and activin membrane-bound inhibitor) were observed during embryogenic development [9–11]. Canonical Wnt signaling pathway has been shown to play a role in fracture repair. This pathway, which activates Lef1/T cell factor (TCF)-dependent transcription, has emerged as a key regulator in embryonic skeletogenesis, positively regulating the osteoblasts. A significant upregulation of β-catenin was found during bone healing process A large molecular array was described to interrelate with each other and with the environment to achieve fracture repair. In this context, regulators of chemotaxis,

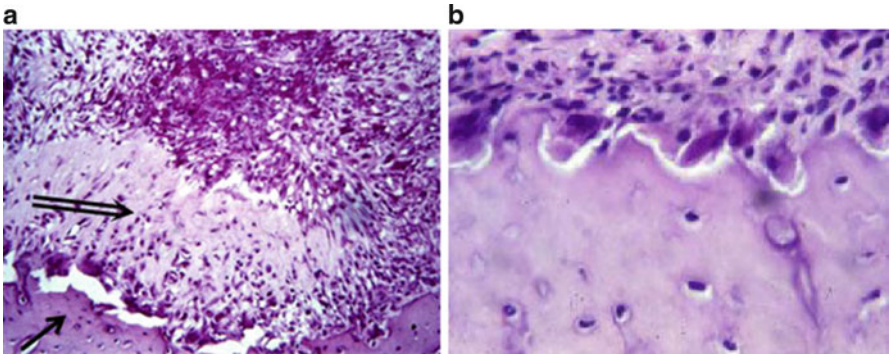

Fig. 1.2 (**a**) Fractured bone (*single arrow*), matrix with osteoblasts (*double arrow*); Note, Granulation tissue (*upper center*). (**b**) Osteoclasts in resorption lacunae (H&E staining)

mitosis, and differentiation such as Wnt, Indian hedgehog, PTHrP genes that respond to hedgehog proteins like Gli 1 and patched (Ptc), platelet-derived GF, matrix metalloproteinases (MMPs), and VEGF a, b, c, and d. Inflammatory cells produce interleukins (IL-1, IL-2, and RANKL). Tumor necrosis factor (TNF x and b) play an essential role [5, 8].

Bone injury is immediately followed by local blood clot formation that serves as a medium that allows cellular migration, proliferation, and capillary budding (Fig. 1.2). Furthermore, the clot was shown to function as a primary source for growth factors [10]. Clot formation is concomitant with the onset of the inflammatory response. At this point, expression of signaling molecules and their proposed functions include IL-1, IL-6, colony-stimulating factors, and TNF-α that play a role in initiating the repair cascade. In addition, TGF-β, PDGF, and BMP-2 expressions increase the initiation of callus formation. Recruitment of mesenchymal stem cells is associated with GDF-8 suggesting its role in controlling cellular proliferation.

It should be emphasized that impaired clotting, due to local or systemic factors, mainly coagulation disorders, anticoagulant drugs and infection, results with a major disruption of healing. The healing process continues with the resorption of the clot and its replacement by granulation tissue. This stage is characterized by an immanent cellular mobilization and vascular in growth from periosteal vessels with extensive neo-angiogenesis mediated by angiopoietins and different VEGFs. A considerable macrophage and osteoclast activity is responsible for the removal and resorption of soft and hard tissue debris by mechanisms mediated by RANKL and MCSF [12–16].

Granulation tissue represents a distinctive pattern of chronic inflammatory reaction, typical to healing in second intention. In bone repair, granulation tissue serves as a transient environment gradually replaced by an ephemeral callus of cartilage and primary bone. Granulation tissue is providing a profuse blood supply and a vehicle for cellular recruitment. At this phase, abundant undifferentiated mesenchymal cells emerge at the site of injury, proliferate, and differentiate, evidently in response to growth factors produced by the injured tissues and from the blood clot. The process