

Bone as a source of organism vitality and regeneration

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Abstract: The most important features that determine the vital role of bone include: a) a continuous supply of calcium, which is indispensable for every cell of the entire organism at all times, and b) the delivery of circulating blood cells and some adult stem cells to keep the body vigorous, ready for self-reparation, and continuously rebuilding throughout life. These functions of bones are no less important than protecting the body cavities, serving as mechanical levers connected to the muscles, and determining the shape and dimensions of the entire organism. The aim of this review was to address some basic cellular and molecular knowledge to better understand the complex interactions of bone structural components. The apprehension of osteoblast differentiation and its local regulation has substantially increased in recent years. It has been suggested that osteocytes, cells within the bone matrix, act as regulatory mechanosensors. Therefore immobility as well as limited activity has a dramatic effect on bone structure and influences a broad spectrum of bone physiology-related functions as well as the functions of many other organs. Lifelong bone rebuilding is modulated through several pathways, including the Wnt pathway that regulates bone formation and resorption. In the adult skeleton, bone is continuously renewed in response to a variety of stimuli, such as the specific process of remodeling dependent on RANK/RANKL/OPG interactions. Better understanding of bone biology provides opportunities for the development of more effective prevention and treatment modalities for a variety of bone diseases, including new approaches to adult stem cell-based therapies. (*Folia Histochemica et Cytobiologica* 2011; Vol. 49, No. 4, pp. 558–569)

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Introduction

The 'Bone and Joint Decade' was officially launched on 13 January, 2000 at the headquarters of the World Health Organization in Geneva. This global campaign was designed to improve the quality of life of people with musculoskeletal conditions such as joint diseases, osteoporosis, osteoarthritis, rheumatoid arthritis, lower back pain, spinal disorders, severe trauma to the extremities, and crippling diseases and deformities in children. The goals were to advance the understanding and treatment of such conditions through research, prevention, and education. The steady in-

crease in life expectancy resulting from advances in medicine leads to an increased prevalence of musculoskeletal disorders, which in turn causes an ever greater socio-economic burden around the world as the population ages. The aims of the campaign were to raise awareness of the increasing societal impact of musculoskeletal injuries and disorders, to empower patients to participate in decisions about their care, to increase funding for prevention activities and research, and to promote research-based cost-effective prevention and treatment [1].

Bone as an organ

The adult human skeleton is composed of 213 bones, excluding the sesamoid bones [2, 3], and makes up about 20 percent of body mass. There are four categories of bones: long bones, short bones, flat bones, and irregular bones. The skeleton serves a variety of

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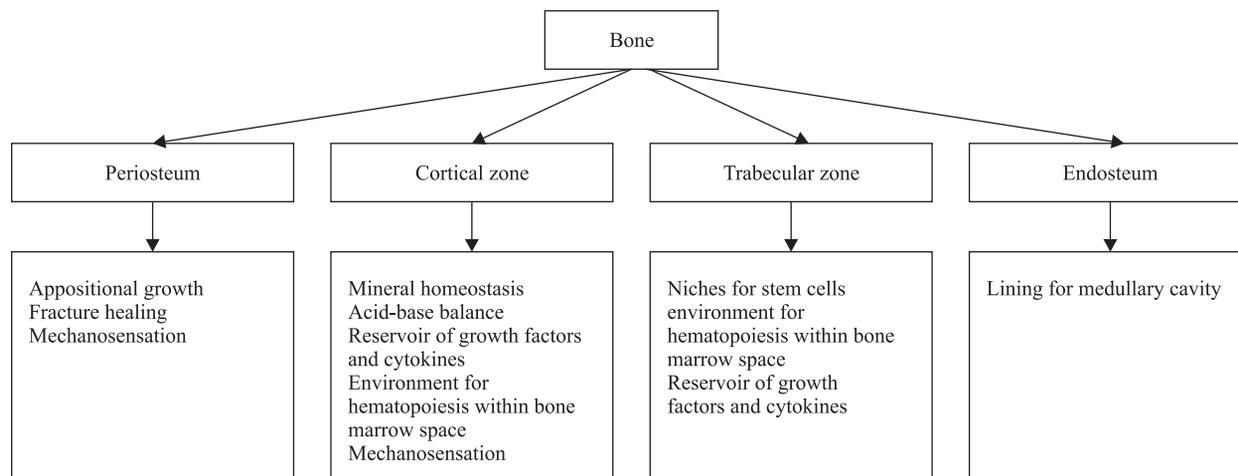


Figure 1. Bone anatomic-functional compartmentalization

functions; the bones provide structural support for the whole body, permit movement and locomotion by providing levers for the muscles, protect internal organs, provide continuous maintenance of mineral homeostasis and acid-base balance, serve as a reservoir of growth factors and cytokines, and provide the environment for hematopoiesis within the bone marrow (BM) spaces [4].

The adult human skeleton is composed of 80% cortical (compact) bone and 20% trabecular (cancellous) bone. Cortical bone has an outer periosteal and inner endosteal surface (Figure 1). Periosteal surface activity is important for appositional growth and fracture repair. Cortical bone and trabecular bone are normally formed in a lamellar pattern, in which collagen fibrils are laid down in alternating orientations. The cortical bone contains osteons (Haversian systems), which are composed of a central canal (Haversian canal) surrounded by lamellae of bone matrix. Within the lamellae, there are osteocytes embedded in tiny spaces — lacunae. The Haversian canal encompasses blood vessels and nerves throughout the bone and communicates with osteocytes in lacunae through canaliculi. The periosteum, consisting of outer and inner fibrous layers, has an osteogenic potential and enables the bone to enlarge [5]. The inside of bone is assembled by a trabecular network (spongiosa) and harbors BM or embryonic connective tissue. The spongiosa ensures elasticity and stability of the skeleton and accounts for the main part (about 70%) of bone metabolism [6].

Bone matrix is mostly composed of type I collagen, trace amounts of types III and V, and FACIT collagens at certain stages of bone formation determining collagen fibril diameter. FACIT stands for Fibril-Associated Collagens with Interrupted Triple Helices, a group of nonfibrillar collagens that serve as molecular bridges important for the organization and stability

of extracellular matrix. Members of this family include collagens IX, XII, XIV, XIX, XX, and XXI. Bone matrix also contains noncollagenous proteins constituting 10% to 15% of total bone protein. Approximately 25% of noncollagenous protein is derived exogenously, including serum albumin and α 2-HS-glycoprotein, which bind to hydroxyapatite. Serum-derived noncollagenous proteins participate in the regulation of matrix mineralization, and α 2-HS-glycoprotein regulates bone cell proliferation. Bone is composed of mineral (50% to 70%) and organic (20% to 40%) matrix, 5% to 10% of water, and < 3% of lipids. The mineral content of bone is mostly hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ with small amounts of carbonate, magnesium and acid phosphate. The remaining organic component consists of non-structural proteins like growth factors, blood proteins, osteonectin and osteocalcin.

One of the important functions of the bone is mechanosensation, though the action of osteocyte-osteoblast/lining cell syncytium. Osteocytes transduce stress signals, induced by bending or stretching of bone, into biologic activity. Flow of canalicular fluid in response to external forces induces a variety of responses within osteocytes. Rapid movements of bone calcium across the filipodial gap junctions stimulate transmission of information between osteoblasts on the bone surface and osteocytes within [7]. Signaling mechanisms involved in mechanotransduction include prostaglandin E2, cyclo-oxygenase 2, various kinases, Runx2, and nitrous oxide. There is a theory [8] that mechanoreceptors in bone can transduce mechanical stimuli into anabolic or catabolic signals for tissues remodeling. Immobility as well as limited activity has a dramatic effect on bone resorption and many organs. This is modulated by major pathways that couple bone formation and resorption, such as the Wnt (Wingless-type MMTV integration site) path-

way [9]. The presence of empty lacunae in aging bone suggests that osteocytes may undergo apoptosis, probably caused by disruption of their intercellular gap junctions or cell–matrix interactions [10]. Osteocyte apoptosis in response to estrogen deficiency or glucocorticoid treatment is harmful to bone structure. Estrogen and bisphosphonate therapy and physiologic loading of bone may help prevent osteoblast and osteocyte apoptosis [11].

Bone remodeling

Bone remodeling begins before birth and continues until the organism's death. In adults about 25% of trabecular and 3% of cortical bone is replaced each year [12]. Bone remodeling increases in perimenopausal and early postmenopausal women and then slows down with further aging of both genders, but continues at a faster rate than in the premenopausal period. Adolescence is a critical time for determining peak bone mass. During this period, bone formation prevails over resorption, and approximately 40% of the total bone mass is accumulated. Several factors affect the accretion of bone mass during adolescence. While genetic predisposition, age, race, and ethnicity cannot be modified, other factors impacting bone such as nutrition, physical activity and lifestyle choices can be altered [13].

All bones in the mammalian skeleton, with the exception of the bones of the calvaria, mandible, part of the maxilla and clavicle are preformed in cartilage moulds from mesenchymal progenitors. Osteoblasts, which differentiate from progenitors in a collar of connective tissue around the middle of the bones where vascular invasion takes place, follow the endothelial cells and lay down bone matrix on the surfaces of these islands of cartilage to form bone struts or trabeculae [14].

Osteoclast precursors (OCPs), derived from progenitors in the spleen and liver, are attracted from blood in the invading blood vessels close to the newly formed bone trabeculae. In an adult organism, osteoclasts are derived from hematopoietic stem cells and share precursors with macrophages, whereas cells of the osteoblast lineage such as stromal cells, bone lining cells, osteoprogenitors, preosteoblasts, osteoblasts and osteocytes are derived from mesenchymal stem cells, which can also differentiate into fibroblasts, chondrocytes, myoblasts and adipocytes. Osteocytes, the terminally differentiated cells of the osteoblast lineage, account for over 90% of all bone cells.

Osteoclastic resorption prevents the development of osteopetrosis, a congenital defect of endochondral ossification, which occurs if osteoclasts fail to form or have impaired activity [15]. As the cartilaginous

centers of the growing bones are removed and replaced by bone and BM, condensations of proliferating and prehypertrophic chondrocytes form close to the ends of long bones, where along with a layer of hypertrophic chondrocytes they constitute the epiphyseal growth plates. This process, called endochondral ossification, requires expression of Runx2, the master transcription factor that regulates bone formation, by mesenchymal osteoblast precursor cells [16]. Hypertrophic chondrocytes express RANKL, OPG and RANK.

Osteoclast formation, activation and resorption are regulated by the ratio of receptor activator of NF- κ B ligand (RANKL) to osteoprotegerin (OPG), IL-1 and IL-6, colony-stimulating factor (CSF), parathyroid hormone, 1,25-dihydroxyvitamin D, and calcitonin [17]. Resorbing osteoclasts secrete hydrogen ions via H⁺-ATPase proton pumps and chloride channels in their cell membranes into the resorbing compartment to lower the pH within the bone-resorbing space to as low as 4.5, which helps mobilize bone minerals. Resorbing osteoclasts secrete tartrate-resistant acid phosphatase, cathepsin K, matrix metalloproteinase 9, and gelatinase from cytoplasmic lysosomes to digest the organic matrix, resulting in the formation of saucer-shaped Howship's lacunae on the surface of trabecular bone and Haversian canals in cortical bone. The resorption phase is completed by mononuclear cells after the multinucleated osteoclasts undergo apoptosis [18]. Resorption is followed by osteoblast activation and formation of osteoid, which fills the cavities over a period of about three months [19].

Bone remodeling is controlled by a system comprising three key participants: RANK, its ligand RANKL, and a decoy receptor OPG. RANK was discovered by Anderson et al. [20] by directly sequencing cDNA from a human BM-derived myeloid dendritic cell. Osteoclastogenesis precedes with the expression of RANKL by bone lining cells, which in turn binds to the RANK that exists as a surface receptor on the membrane of preosteoclasts. The receptor–ligand binding promotes an intricate and distinct signaling cascade for osteoclast activation and commitment [21, 22]. The expression of RANKL is up-regulated in the presence of interleukin-1 (IL-1), tumor necrosis factor α (TNF- α) and vitamin D, whereas transforming growth factor β (TGF β) and estrogens have an opposite effect.

Understanding the osteoclast activation process is one of the most important discoveries in bone biology of recent years [23]. RANKL is a member of the tumor necrosis factor family, and is the most important cytokine involved in the final stages of osteoclast maturation and activity. The co-ordinated action of bone cells is designated as the Basic Multicellular Unit (BMU). Although there are many systemic factors that initiate osteoclastogenesis, they all appear to work

via the final common pathway by increasing production of RANKL by osteoblasts [23]. The action of RANKL on osteoclasts is opposed by the soluble receptor OPG, which belongs to the tumor necrosis factor (TNF) receptor family [23] and is secreted by osteoblasts and stromal cells. Cells of the osteoblast lineage control the formation and activity of osteoclasts, which, in turn, are responsible for the initiation and execution of resorption at remodeling sites. There is a period of bone formation mediated by osteoblasts, followed by full mineralization of the newly formed bone matrix [24]. Bone formation takes approximately 4–6 months to complete. Osteocytes, the terminally differentiated osteoblasts, become embedded in the osteoid matrix in which they were laying down.

Discovery of the RANKL/RANK/OPG system has been one of the most important advances in bone biology in recent years. This signaling system is essential for skeletal homeostasis, and its disruption leads to changes of bone resorption *in vitro* and in animal models [25–27] of most bone diseases. OPG protects bone from excessive resorption by binding to RANKL and preventing it from binding to RANK [28]. OPG acts as a decoy receptor [29]. It is known that there is an ongoing bone formation despite the absence of functional osteoclasts and reduced bone resorption [30]. A hypothetical agent that could inhibit bone resorption but allow continuous bone formation would have a greater effect on bone mass and bone quality than any currently available agent.

RANKL is a homotrimeric, typically membrane-bound, protein present on osteoblastic and activated T cells. It can be also secreted by some cells, such as activated T cells [31]. Most of the factors known to stimulate osteoclast formation and activity induce RANKL expression by osteoblastic stromal cells. RANKL expression increases in response to a variety of pro-resorptive signals such as proinflammatory cytokines, glucocorticoids, estrogen deficiency and PTH excess [32]. RANKL binds to the RANK receptor which is expressed on osteoclasts and their precursors. RANKL is a critical stimulator of the differentiation and activity of osteoclasts and thus it plays a critical role in the promotion of bone resorption.

OPG is a decoy receptor for RANKL that is secreted by osteoblasts, and to a lesser extent by other stroma-derived cells, including those in the heart, kidney, liver, and spleen. Most of the factors that induce RANKL expression by osteoblasts also regulate OPG expression [33]. Osteoclast numbers and its activity can increase in response to a higher RANKL/OPG ratio. A change in both leads to a change in favour of RANKL. The discovery of RANKL, RANK and OPG has led to the development of specific inhibitors of RANKL, some of which, such as OPG and a mono-

clonal antibody to RANKL, have been tested in humans in clinical trials with successful inhibition of bone resorption.

Upon the completion of bone resorption, resorption cavities contain a variety of mononuclear cells, including monocytes, osteocytes released from bone matrix, and preosteoblasts recruited to begin new bone formation. Coupling signals to induce new bone formation include bone matrix-derived factors such as TGF- β , IGF-1, IGF-2, bone morphogenetic proteins (BMPs), PDGF, or fibroblast growth factor. The phase of bone resorption is mediated by the strain gradient in the lacunae [34].

Osteoblasts synthesize new collagenous organic matrix and regulate mineralization of matrix by releasing small, membrane-bound matrix vesicles that concentrate calcium and phosphate, and enzymatically destroy mineralization inhibitors such as pyrophosphate and proteoglycans. Osteoblasts surrounded by and buried within matrix become osteocytes. They form an extensive canalicular network with bone surface lining cells, osteoblasts, and other osteocytes, maintained by gap junctions between the cytoplasmic processes extending from the osteocytes. The osteocyte network within the bone serves as a functional syncytium. At the completion of bone formation, approximately 50% to 70% of osteoblasts undergo apoptosis, while a smaller proportion become osteocytes or bone-lining cells. Bone-lining cells may regulate the influx and efflux of mineral ions in and out of bone extracellular fluid, thereby serving as a blood-bone barrier. They retain the ability to re-differentiate into osteoblasts upon exposure to parathyroid hormone or mechanical forces. Bone-lining cells within the endosteum lift off the surface of bone before bone resorption to form discrete bone remodeling compartments with a specialized microenvironment [35]. The remodeling process is essentially the same in cortical and trabecular bone.

The intensity of bone resorption depends on osteoclast secretion of hydrogen ions and cathepsin K enzyme. H⁺ ions acidify the resorption compartment beneath osteoclasts to dissolve the mineral component of bone matrix, whereas cathepsin K digests the proteinaceous matrix. Osteoclasts bind to bone matrix via integrin receptors in the osteoclast membrane linking to bone matrix peptides. The β 1 family of integrin receptors in osteoclasts binds to collagen, fibronectin and laminin, but the main integrin receptor facilitating bone resorption is the α β ₃ integrin. The ligands for the α β ₃ integrin include osteopontin and bone sialoprotein [36].

Binding of osteoclasts to bone matrix causes their polarization. The bone resorbing surface develops a ruffled border when acidified vesicles that contain matrix metalloproteinases and cathepsin K are trans-

ported via microtubules to fuse with the membrane. The ruffled border secretes H^+ ions via H^+ -ATPase and chloride channels and causes exocytosis of cathepsin K and other enzymes in the acidified vesicles. Ruffling of the cytoplasmic membrane increases the area of the cell surface for secretion of the proteolytic enzyme, cathepsin K, and hydrochloric acid [37]. By the sealing and secretory mechanism, osteoclasts simultaneously degrade the matrix and dissolve the mineral of bone, while protecting neighboring cells from the harmful effects of HCl. Actively resorbing osteoclasts form podosomes that allows them to be firmly attached to bone matrix, rather than being focally adherent, as with most other cells. Podosomes are composed of an actin core surrounded by $\alpha_3\beta_3$ integrins and associated cytoskeletal proteins.

The rate of bone remodeling and the number of remodeling sites are increased in a variety of pathologic conditions affecting the skeleton, including postmenopausal osteoporosis, hyperparathyroidism and rheumatoid arthritis. In these disorders, the local and/or systemic alterations in the levels of hormones or pro-inflammatory cytokines stimulate bone resorption [38]. The induction of bone resorption is regulated predominantly by indirect mechanisms that involve upregulation of the expression of M-CSF and RANKL by osteoblastic and other cells. Osteoclasts are not simply bone resorbing cells, but they also regulate osteoblast functions, mediate the egression of hematopoietic stems from the marrow into the blood [39], and function as immunomodulators in pathologic states [40].

Calcium reservoir

Bone is regarded as a mineral reservoir releasing calcium and phosphate in response to hormones secreted from remote organs [41]. Significant elements of bone are Ca^{2+} and P_i [42]. Precise control of plasma calcium (Ca^{2+}) and phosphate (P_i) levels is essential to the performance of many vital physiological functions [43]. Muscle contraction, blood clotting and neuronal excitation all require Ca^{2+} , whereas P_i , as a component of membrane lipids and backbone of DNA, is crucial to intracellular signaling. Several organs contribute to the exquisite regulation of Ca^{2+} and P_i homeostasis by facilitating intestinal absorption, bone (de)mineralization, and renal excretion/reabsorption. Regulation of these processes occurs by a number of hormones, including the biologically active form of vitamin D (1,25-dihydroxyvitamin D_3), parathyroid hormone (PTH), and calcitonin. Fibroblast growth factor member 23 (FGF23) has also been identified as essential in the regulation of Ca^{2+} and P_i homeostasis [44]. The vast majority of whole body

Ca^{2+} and P_i is stored as the mineral hydroxylapatite in the skeleton. In blood, 45% of Ca^{2+} is present in a free, ionized form, 45% is bound to proteins, and a small fraction, 10%, forms complexes with citrate, sulfate, and phosphate anions. PTH, $1,25(OH)_2D_3$, and estrogen exert their effect on renal-mediated Ca^{2+} handling by altering, at the transcriptional level, the expression of Ca^{2+} transporters [45]. PTH maintains a physiological balance of calcium and phosphate concentrations by binding to its receptor on the plasma membrane of cells in bone and kidney. It signals through multiple pathways, including protein kinases A and C, although a preference for certain pathways is apparent in each organ and function [46]. The calcium-sensing receptor is a G-protein-coupled, seven-pass transmembrane molecule present in the parathyroid gland. Kidney is a key site to co-ordinate calcium homeostasis by regulating the release of PTH from the parathyroid glands [47].

It is known that the osteocyte and bone lining cells, under certain physiologic conditions, can participate in mobilizing calcium from the skeleton to maintain calcium balance [48–51]. When mineralization is required, tissue non-specific alkaline phosphatase, an enzyme associated with skeletal and cartilage mineralization, cleaves orthophosphates from polyphosphates. The hydrolytic degradation of polyphosphates in the calcium-polyphosphate complex increases orthophosphate and calcium concentrations and therefore it favours apatite mineral formation. The correlation of alkaline phosphatase with this process may be explained by the destruction of polyphosphates in calcifying cartilage and areas of bone formation [52].

Bone as a source of adult stem cells

Under specific environmental conditions, self-renewing (Figure 2), pluripotent stem cells give rise to osteoprogenitor cells in various tissues. BM contains a small population of mesenchymal stem cells, distinct from the hematopoietic stem cell population that gives rise to blood cell lineages that are capable of giving rise to bone, cartilage, adipose, or fibrous connective tissue. Commitment of these mesenchymal stem cells to the osteoblast lineage requires the activation of the Wnt/ β -catenin pathway and associated proteins [53]. The Wnt system is also important in chondrogenesis and hematopoiesis and may be stimulatory or inhibitory at different stages of osteoblast differentiation.

Cell-based therapy (Figure 3) could soon become a new strategy to treat a wide array of clinical conditions. The use of adult stem cells has major advantages: (a) adult stem cells can be isolated from patients, thus overcoming the problems with immunological rejection, and (b) the risk of tumor formation

is greatly reduced compared to the use of embryonic stem cells [54].

Adult stem cells are clonogenic, self-renewing, and pluripotent cells with a plasticity to differentiate into cell types of the particular tissue in which they reside, and often to trans-differentiate into different types of tissues [55]. Adult stem cells are usually located in a specific cellular niche, which determines the status of stem cell activation, ensuring a balance between

maintenance of the stem cell pool and production of progenitor cells engaged in tissue differentiation [56]. Hematopoietic stem cells (HSCs), which can be isolated from BM, are among the best characterized adult stem cells and the stem cells being currently used in the clinics. Some other stem cells are also used, but in the preclinical trials. BM also accommodates stromal cells, mesenchymal stem cells, and various blood cells at different stages of maturation, as well as their progenitors. HSCs constitute only a small fraction of BM population (1 in 10⁴ to 1 in 10⁸ of BM nucleated cells) [57]. HSCs are able to renew themselves or differentiate into precursors, which produce specialized hematopoietic cells, including lymphocytes, dendritic and natural killer cells, megakaryocytes, erythrocytes, granulocytes, and macrophages [58]. Cells in the hematopoietic hierarchy have diverse differentiation potential and self-renewal capacities, and are able to cope with the high demand to continuously produce large numbers of blood cells. The first progeny of HSCs are multipotent progenitors that retain the ability to differentiate into all hematopoietic lineages but show a lower capacity to proliferate [59]. Human HSCs are known to exhibit CD34⁺, Thy1⁺, CD38^{low/-}, C-kit^{low}, CD105⁺, Lin⁻ phenotype [60, 61].

In 1998, Ferrari et al. [62] reported that mouse bone-marrow-derived cells give rise to skeletal muscle cells when transplanted into damaged mouse

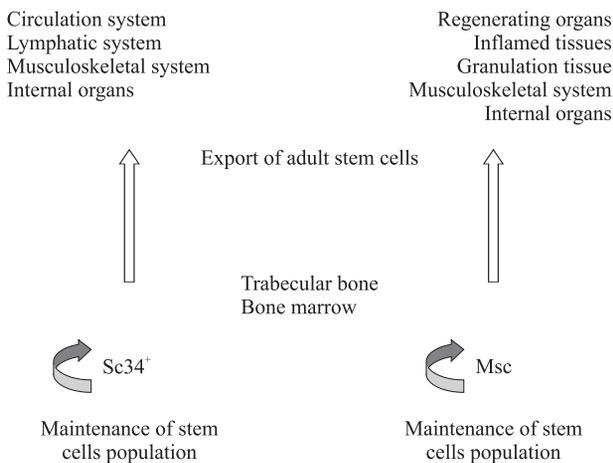


Figure 2. Bone marrow as a source of adult stem cells. Sc34⁺ — hematopoietic stem cells; Msc — mesenchymal stem cells

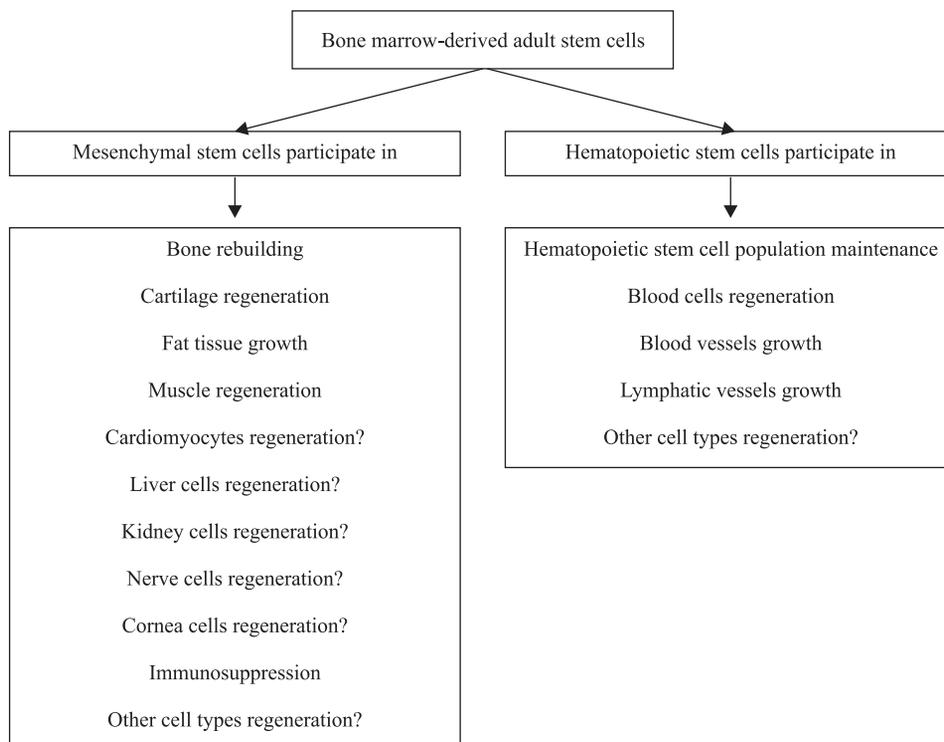


Figure 3. Bone marrow-derived adult stem cells participation in organs regeneration

muscle. Thereafter, transplanted bone marrow cells were reported to generate a wide spectrum of different cell types, including hepatocytes [63], endothelial, myocardial [64–66], neuronal, and glial cells [67, 68]. Moreover, HSC can produce cardiac myocytes and endothelial cells [69], functional hepatocytes [70], and epithelial cells of the liver, gut, lung, and skin [71]. Mesenchymal stromal cells (MSC) of the bone marrow can generate brain astrocytes [72] and some other cell types. Nevertheless, the transformation of transplanted bone marrow cells is still a matter of debate, and pluripotency of these cells has not been convincingly demonstrated in many cases.

Different types of stem cells can be combined at progenitor-committed stages, thus greatly enhancing the therapeutic outcome [73] and ultimately leading to the rejuvenation of the whole organ.

Understanding the basic molecular mechanisms underlying cell fate switching of adult stem cells will be essential to ensure their safe use in regenerative medicine. In the near future, it will most likely be possible to transplant genetically modified stem cells that carry a set of genes critical for their various functions, e.g. trans-differentiation, that are under externally regulated promoters [74] and, depending on the therapeutic requirements, direct their differentiation into desired cell populations.

Recent studies on the plasticity of murine myotubes [75] and other cells derived from adult tissues suggest that dedifferentiation may be possible in mammals [76]. At the molecular level, MSX1 (AKA Msh homeobox protein) has been identified as a possible factor involved in dedifferentiation processes in human cells [75, 76]. Another small molecule, reversine, has been shown to induce murine myogenic lineage-committed cells to become multipotent mesenchymal progenitor cells that can proliferate and re-differentiate into bone and fat cells [77]. Epigenetic cell changes are probably involved and may be mediated by signals received from the injured cells.

Adult stem cells have the advantage of being obtainable using relatively noninvasive, autologous harvest methods. They are also the most promising choice for the majority of clinical purposes. Bone marrow stromal cells have attracted the attention of many scientists interested in cellular strategies in the repair of neural tissue [78–81]. These mesenchymal stem cells are harvested from the long bones, and when placed in culture medium containing the appropriate cytokine cocktail, transdifferentiate into Schwann cell-like phenotype [82].

The term multipotent mesenchymal stromal cell (MSC) has recently been coined to describe this cell type [83, 84], although, in the majority of scientific

publications, they have been generally referred to as mesenchymal stem cells [85]. Adult mesenchymal stem cells can be isolated from bone marrow or marrow aspirates, and because they are culture-dish adherent, they can be expanded in culture while maintaining their multipotency. MSCs have been used in preclinical models for tissue engineering of bone, cartilage, muscle, marrow stroma, tendon, fat, and other types of connective tissues [86]. MSCs secrete a broad spectrum of bioactive macromolecules that are both immunoregulatory and serve to structure regenerative microenvironments in fields of tissue injury. Horwitz et al. have reported the use of MSCs for the repair of bone in patients with osteogenesis imperfecta [87].

Bone as a source of self restoring and healing

Every cell in the body has a specific half-life. Every cell reaches its maturity and subsequently dies. Red blood cells have half-lives of 60–90 days and arise in a multi-step lineage from the hematopoietic stem cell (HSC). The progenitor cells furnish the replacement units for normal cell death. The normal half-lives of mature cells thus provide a mechanism for rejuvenating living tissue with fresh, functional cell units [86]. This allows the replacement of cells that could be non-functional, contain mutations, and substitute with cells slightly different from the expired cells. These changes over time are referred to as aging. Adult MSCs are responsible for the replacement of osteoblasts that in humans have half-lives of 8–10 days. Loss of bone mass results from the diminution of regenerative units in the marrow. The capacity of culture-expanded marrow-derived MSCs to differentiate into bone, cartilage, etc. is independent of the age of the donors. Bone density and bone mass are dependent on the conversion of MSCs into osteoblasts that fill the pits of osteoclast-resorbed bone. The niche where MSCs actually reside in marrow is not known.

Since MSCs can differentiate into distinctive mesenchymal phenotypes, they have been used to restructure tissues when encased in tissue-specific scaffolds and implanted into different tissue sites. For example, in rodents, dogs and humans, autologous marrow MSCs have been delivered to long-bone repair sites in calcium phosphate porous ceramics to produce morphologically and biomechanically superior bone [88]. Others have used marrow MSCs in hyaluronan and polymeric scaffolds for cartilage repair [89]. At least three different methods have been employed for using MSCs in scaffolds. MSCs have been loaded into the scaffolds *in vitro* and, after a short incubation to insure attachment, the cell-scaffold composites were implanted. Another way is to incubate the

cell-scaffold composite in differentiation medium to stimulate MSC progression into a specific lineage, and after 7–14 days, the composite is implanted into orthotopic sites [90]. The last approach is to implant scaffolds, to which targeted cells are able to attach to docking sites, or to implant scaffolds with the included cells in protective coats, and allow the scaffold to mature *in vivo* [91]. All of these techniques have resulted in well-integrated, newly differentiated bone tissue. These approaches have been used in various animal models and in limited numbers in human beings [88]. However, no human MSC-based tissue engineering technology is currently clinically available.

MSCs secrete bioactive factors that inhibit scarring and apoptosis, and stimulate angiogenesis and mitosis of tissue-intrinsic stem and progenitor cells. This complex, versatile activity due to the secretory activity of MSCs is known as ‘trophic activity’ [86].

Mesenchymal stromal cells show great promise to become biological therapeutic agents for a diverse range of medical needs. Natural chemo-attractive mechanisms result in the recruitment of MSCs from remote areas to sites of tissue damage in order to establish a reparative/regenerative microenvironment. The age of the individual patient, the extent of tissue damage, and the local and whole body quantity of MSCs probably play a role in the rate and extent of the repair and/or regeneration of damaged tissue. Various techniques of direct delivery or manipulative targeting of MSCs to sites of tissue damage may, in future, lead to profound control of damage, cell death, scarring, and subsequent regeneration of various tissues.

Total joint replacement of the upper and lower extremities is one of the most efficacious, cost effective interventions in orthopaedic surgery. This type of procedure alleviates pain and improves function in a consistent fashion. Immediately after the implantation, articulating and non-articulating surfaces begin to wear and generate wear particles. These particles are generally distributed locally within the joint itself; they often may be found in the regional lymph nodes, and in some cases systemically. Periprosthetic osteolysis may undermine the bone bed and compromise the stability of the implant. Idiosyncratic immune reactions may also occur.

Over the weeks and months following a joint replacement, the trabecular callus surrounding implants undergoes successful osseointegration remodeling to form a more consolidated mature structure that can transmit load more effectively. Stable implants that abut cortical bone can osseointegrate directly via mesenchymal osteoprogenitor cells from the marrow and endosteum [92]. Stable implants with a fibrous or cartilagenous encapsulation layer can undergo so-

called ‘primary bone healing’ directly, with metaplasia of the encapsulation tissue to bone.

MSCs are potentially immunoprivileged; therefore their implantation in an allogeneic setting has been used to facilitate tissue repairs for bone and cartilage defects. Cell therapy using adult stem cells is also a potential approach for treating degenerative disk disease [93].

Bone-related pathology

Bone loss results from the direct effects of inflammation, poor nutrition, reduced lean body mass, immobility, and the effects of treatments, especially with glucocorticoids [94]. Inflammatory disease can increase bone resorption and decrease bone formation, but most commonly it impacts on both of these processes, resulting in an uncoupling of bone formation from resorption in favour of excess resorption.

Glucocorticoids are commonly used to treat many inflammatory diseases. They frequently have major adverse effects on bone that is difficult to separate from the effects of inflammation itself. Inflammation also impacts the control of reproductive hormones, leading frequently to hypogonadism in both men and women. Inflammation influences the secretion and action of PTH, which can lead to an increase in bone resorption. In the adult skeleton, the combination of many factors, such as the rate of bone turnover, collagen matrix, structure, geometry, and density, determine the bone’s overall mechanical competence. Defects in these parameters can result in diseases such as osteoporosis, osteopetrosis, osteogenesis imperfecta, and Paget’s disease [24].

Consistent with the anabolic effects of mechanical stimuli, the reduction or removal of mechanical loads results in bone atrophy, altering the mass, morphology, cellular activity, and its material properties. In humans, much of the understanding of disuse-induced bone loss comes from investigations documenting the effects of space flight (microgravity) [95–97] and prolonged bed rest [96, 98, 99]. During space travel, the removal of gravitational and most functional loads triggers pronounced bone atrophy, with astronauts losing bone mineral at a rate of approximately 1–2% per month [97]. The atrophy is site-specific, with greater decay generally observed in the lower appendicular skeleton than the spine, and type-specific, with trabecular bone removal three to five times greater than that of cortical bone [98]. Bed rest studies have yielded similar results, showing that the bone mineral density (BMD) of healthy males confined to bed rest for 17 weeks decreases by 0.9–1.3% per month in the tibia, femur, and lumbar spine [98, 100].

During functional loading, the complete strain state of any given piece of bone tissue is typically very complex, but it can be described in general terms by two predominant components: normal strains cause volumetric changes in the tissue, while shear strains cause angular deformations.

When changes in remodeling events are compared between loading regimes inducing predominantly shear or predominantly normal strains, it becomes clear that bone tissue can readily differentiate between different kinds of deformation; even though bone cells are responsive to both normal and shear strains, only normal strains increase the degree of intracortical turnover [101]. The development of effective biomechanical interventions in areas such as orthodontics, craniofacial repair or osteoporosis will require the identification of the specific components of the bone's mechanical environment that are anabolic, catabolic, or anti-catabolic [102].

Bone responds to a great variety of mechanical signals. Both high- and low-magnitude stimuli can be sensed by the skeleton. The ability of physical signals to influence bone morphology is strongly dependent on the signal's magnitude, frequency, and duration. Bone's sensitivity to the application and removal of mechanical signals is under tight control of the genome. Bone loss modulated by the removal of weight-bearing activities is profoundly influenced by a number of factors such as genetics, gender, and baseline morphology.

The bone mass loss associated with aging can be reduced by the implementation of certain exercise programs in adults and the elderly [103]. Several training methods have been used in prospective studies to improve BMD. Not all exercise modalities have shown positive effects on bone mass. For example, unloaded exercise such as swimming has no impact on bone mass, while walking or running has only a limited positive effect. On the other hand, even a relatively small amount of high impact exercise appears to be the most efficient for enhancing bone mass, although this has not been proven in postmenopausal women. Impact and resistance exercise should be advocated for the prevention of osteoporosis. To reduce the likelihood of falling and its associated morbidity and mortality of patients with osteoporosis, weight-bearing exercise in general, and resistance exercise in particular, along with exercises targeted to improve balance, mobility and posture, should be recommended.

Obesity is protective for the skeleton, whereas low body weight in elders is a major risk factor for fractures [104]. The hypothalamus modulates fat and bone via the sympathetic nervous system by regulation of appetite, insulin sensitivity, energy use, and skeletal remodeling. In the bone marrow, fat and bone cells arise from the same stem cells.

Osteoporosis, a severe bone loss in which bones become increasingly porous and easy to fracture, has become one of the major health and socio-economic problems in many countries. It is estimated that more than 200 million people worldwide (1 in 3 women and 1 in 12 men over the age of 50) suffer from osteoporosis, with three to four times as many being at risk because of low bone mass [105]. Osteoporosis-related fractures often begin a downward spiral in health and independence for the elderly.

Osteoporosis is a classical age-related disease that affects women more often than men [106]. Estrogen deficiency has direct, as well as indirect, impact on bone metabolism. Osteoporosis is common, costly, and morbid. Its prevalence is increasing as the population ages.

Our understanding of osteoblast differentiation and local regulation has increased over recent years through the discovery of the Wnt signaling pathway and its antagonists. The Wnt family of glycoproteins represents a major signaling pathway that is involved in cellular differentiation [107].

Scientific understanding of osteocytes and their role in bone metabolism has significantly increased in recent years. The osteocyte is a nonproliferative, terminally differentiated cell of the osteoblast lineage [108]. It is the most abundant cell type in bone, and resides in the lacuna/canalicular system. There is strong evidence supporting its role in the control of local bone remodeling. The surface area of the lacuna/canalicular system is large — more than 100 times that of the trabecular bone surface. The canalicular system of communication for the osteocytes is similar to that of the nervous system. It is composed of a large number of low activity cells connected through the canaliculi, which, it is hypothesized, serve as an efficient way to transmit signals over long distances. The osteocytes are surrounded within their lacunae by proteoglycans that assist in the amplification of fluid flow-derived mechanical signals. Each osteocyte has a cilium extending from its cell cytoplasm, which may also translate the fluid flow signal to the osteocyte. It has long been known that mechanical stress induced by weight-bearing exercise increases osteoblast activity.

The stimuli responsible for the osteocyte's action have not been fully characterized. Recently discovered sclerostin is an example of an osteocyte-derived protein that plays an important role in the inhibition of bone formation. Sclerostin is one of the Wnt signaling antagonists known to inhibit osteogenesis [109]. This aspect of osteocyte biology may be very important for the development of novel anabolic agents to treat osteoporosis. The current therapies for osteoporosis include anti-resorptive treatment such as

bisphosphonates, hormone replacement, and selective estrogen receptor modulators [11]. The search for new anabolic therapies is focused on biological pathways involved in the stimulation of the osteoblast lineage cells to differentiate from a progenitor cell, to proliferate faster, and to produce more organic matrix proteins. Several signaling factors, including BMPs, PTH, endothelin, fibroblast growth factors, steroidal hormones, insulin-like growth factors, and prostaglandin agonists have emerged as crucial in the regulation of osteoblast maturation, but the Wnt family of ligands has arguably generated the most interest and excitement in recent years. Wnts are cysteine-rich, secreted glycoproteins that activate cell-surface-receptor-mediated signaling pathways to control gene expression, cell fate determination, proliferation and migration. Modulation of Wnt signaling pathways has emerged as a promising and feasible strategy to increase bone density [110, 111].

Nuclear hormone receptors represent a major group of regulatory factors that control critical processes of bone growth and metabolism [34, 112]. It is well known that male bones have higher mineral density and a lower risk of fracture or osteoporosis than female bones [113]. A decrease in testosterone level is associated with many symptoms and signs of aging, such as decrease in muscle mass and strength, cognitive decline, reduction of bone mass, and increase in (abdominal) fat mass [114].

Although genetic predisposition, age, race, and ethnicity as influences on bone structure cannot be modified, other factors such as nutrition, physical activity, and life style choices can [13]. Promoting a healthy lifestyle, regular weight-bearing physical activity, and a healthy diet with optimal calcium and vitamin D intake has been proved to be the effective way to achieve a maximal peak bone mass.

Conclusion

This review highlights the developments in bone biology in recent years. We believe that in the near future, scientific advances in this field will lead to improvements in the prevention and treatment of many bone diseases, and will expand the bone marrow-derived adult stem cell-based therapy into more diseases.

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