Chapter 2

General Introduction

1. Introduction

Bone is a remarkable tissue with diverse functions. In close collaboration with ligaments, muscles, and tendons the skeleton literally forms the backbone of locomotion. In addition, the skeleton protects vital organs, including bone marrow, brain, spinal cord, heart, and lungs. And last but not least, bone has a metabolic function, acting as a reservoir of ions, especially calcium and phosphate, for the maintenance of serum homeostasis. Bone is a highly specialized connective tissue. The skeletal system consists of bone and cartilage. The rigidity of bone tissue relies on mineralization of the bone matrix with hydroxyapatite crystals, which are predominantly composed of $Ca_{10}(PO_4)_6(OH)_2$. There are two major families of bone cells, the osteoblast and the osteoclast lineages.[179,180] Osteoblasts and osteoclasts are responsible for the dynamic turnover of bone both during growth and in adult life. The osteoblast is the bone-lining cell responsible for the production of bone matrix, whereas the osteoclast is a multinucleated bone-lining cell responsible for bone resorption. During bone formation osteoblasts are embedded within the bone matrix in small lacunae to form osteocytes (Fig 1).

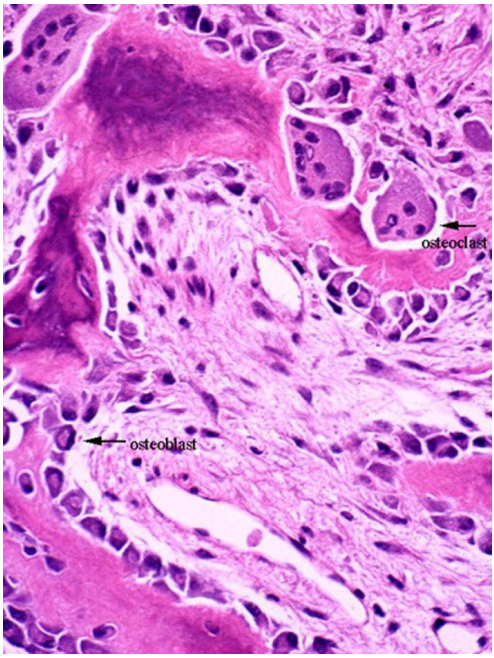

Fig 1. Osteoblasts are present on the lower left, lining a bone trabecula. Osteocytes can be seen embedded within the bone matrix. Osteoclasts are present at the right top corner.

Bone formation occurs through the coordinated production and mineralization of the osteoid matrix. Bone matrix components consist of three classes of macromolecules. The first group possesses repetitive structural motifs and includes collagen, hyaluronan, decorin and biglycan. The second group is characterized by a modular domain structure and includes versican, thrombospondin, fibronectin, osteonectin, and tenascin. The third group demonstrates no clear structural motifs and includes matrix γ-carboxylated protein, bone sialoprotein, osteopontin, and osteocalcin.[139] The complexity of the interactions between these various matrix components and bone cells conveys to bone its unique function of sustaining a stable yet dynamic structure. The major organic component of the extracellular bone matrix is type I collagen, accounting for up to 90% of the organic matter. Collagen I is composed of trimers of two α1 chains and one α2 chain, to form triple helical molecules. The collagen α chains are produced as procollagen which possesses amino- and carboxyl-terminal polypeptide extensions. Enzymatic removal of the noncollagenous N- and C-terminal extensions precedes collagen fibril formation. Collagen III is also found in bone matrix, but its role in bone metabolism is unclear.

2. Bone histology and histiogenesis

On gross observation of bone, cancellous and compact bone can be distinguished. Cancellous bone consists of many trabecular walls separating numerous interconnecting cavities filled with bone marrow or fat tissue. Compact bone does not show these cavities. Nevertheless, on microscopic examination both cancellous and compact bone have the same basic histologic structure. In long bones, compact bone is mainly found in the diaphysis whereas cancellous bone is located in the metaphyseal areas and the epiphysis, surrounded by a thin layer of compact bone.[192] Flat bones of the skull, scapula, and pelvis usually have a core of cancellous bone flanked by two plates of compact bone. On histology, there are two varieties of bone tissue: primary, immature or woven bone; and secondary, mature or lamellar bone. The difference between the two relies on the fact that collagen bundles are placed randomly in the first variety and organized into bone lamellae in the second.

Primary bone is the first bone tissue formed during bone formation. It is temporary and is readily replaced by secondary bone tissue in most places in the skeleton. In addition to the irregular deposition of collagen bundles, primary bone is characterized by a lower amount of mineralization and a larger amount of osteocytes compared to secondary bone.

Secondary bone is the variety mainly found in the mature skeleton. The bone lamellae, which characterize mature bone, are arranged parallel to each other or concentrically surrounding a central canal containing blood vessels and nerves. This complex of concentric lamellae is called the osteon or Haversian system and is the main building block of the skeleton (Fig 2). Between, and occasionally within the lamellae, lacunae containing osteocytes are encountered. These osteocytes have numerous long cell processes, which are in contact with other osteocytes and osteoblasts. Osteocytes are still capable of matrix production. The osteons communicate with each other, the endosteum, the marrow cavity, and the periosteum through transverse or oblique Volkmann's or perforating canals (Fig 2). These Volkmann's canals have no concentric lamellae and perforate the lamellae of the osteons. During growth and also in the adult skeleton continuous remodeling of osteons takes place. This explains the great variability in size and form of osteons.

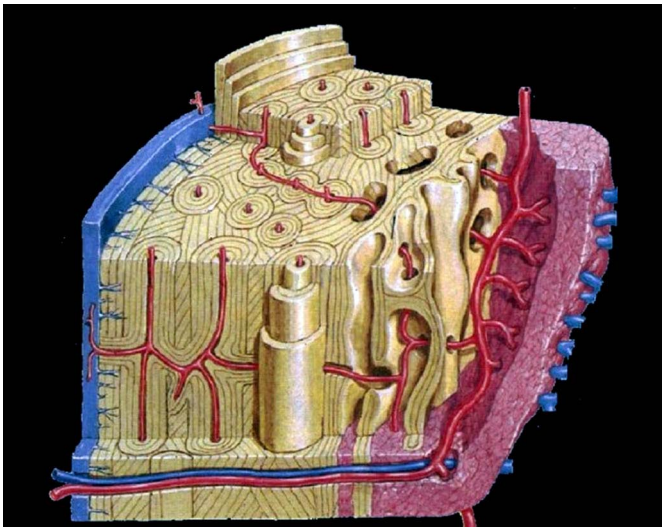

Fig 2. Representation of bone histology demonstrating the main building unit of bone i.e., the osteon.

Bone tissue is formed either by intramembranous ossification or by endochondral ossification.[87] Intramembranous ossification is characterized by direct bone formation within a layer or membrane of connective tissue i.e., periosteum. During endochondral ossification, a cartilaginous model precedes the actual accretion of bone tissue. In both intramembranous and endochondral ossification bone tissue is deposited first as primary or immature bone. Through

remodeling this primary bone is replaced by mature lamellar bone. Most of the bones of the skull, including parietal bones, frontal bones, mandible, and maxilla are formed by intramembranous ossification. Growth in width of short and long bones proceeds through intramembranous bone formation. Primary ossification starts within the connective tissue layers. Preosteoblasts differentiate into osteoblasts, which start producing osteoid.[179] Osteoid is mineralized in turn to form primary bone. The trabeculas of primary bone unite to create cancellous bone. Growing blood vessels and undifferentiated mesenchymal cells, which give rise to bone marrow cells, including preosteoclasts, penetrate the cancellous bone.

Endochondral ossification is responsible for the formation of most short and long bones. Endochondral ossification relies on the replacement of a hyaline cartilage and basically depends on two processes. The first process is hypertrophy and apoptosis of chondrocytes of the model of bone leaving expanded lacunas separated by septa of calcified cartilage matrix. In the second process, an osteogenic bud consisting of blood capillaries and osteogenic precursor cells penetrates into the lacunas left by the apoptotic chondrocytes. The undifferentiated cells give rise to osteoblasts, which lay down osteoid on the remnants of the calcified cartilage matrix. In this way, bone tissue appears at the site where there was cartilage.

Long bones are formed from cartilaginous models with a cylindrical shaft or diaphysis and enlarged extremities or epiphyses. The first bone to develop in the diaphysis is the bone collar, which surrounds the periphery of the cartilaginous matrix and thus forms the shaft of the bone. This bone collar is produced through intramembranous ossification. Within the forming bone collar, chondrocytes of the cartilage model start the process of hypertrophy, apoptosis, lacuna formation, and mineralization of the remaining cartilage matrix, also known as hypertrophication. Blood vessels of the osteogenic bud invade the lacuna and osteoblasts start to synthesize bone matrix. This ossification center, which appears in the diaphysis, is called the primary ossification center. At later stages of development a secondary ossification center arises at the end of the long bone to form an epiphysis or apophysis. Instead of the longitudinal growth of the primary ossification center, growth in the secondary center is radial. As bone formation in the primary and secondary ossification centers progresses, an epiphyseal plate or growth plate is formed between the diaphysis and epiphysis.[89] By analogy an apophyseal plate is formed between the diaphysis and apophysis. During adolescence, longitudinal bone growth continues in the growth plate through a highly coordinated type of endochondral ossification.[21]

In the epiphyseal plate five zones can be distinguished. Starting from the epiphyseal side these are: 1- the resting zone containing hyaline cartilage and small chondrocytes; 2- the proliferative zone with rapidly dividing chondrocytes, which

form columns of stacked cells parallel to the long axis on the bone; 3- the hyperthrophic cartilage zone with enlarged chondrocytes and interspersed thin septa of resorbed cartilaginous matrix; 4- the calcified cartilage zone with chondrocyte apoptosis. The cartilaginous matrix septa are mineralized with hydroxyapatite; 5- the ossification zone in which primary bone is formed. Blood vessels and osteoblasts invade the calcified cartilage matrix and deposit osteoid on the septa. The osteoid is mineralized, thus forming primary bone tissue. Longitudinal bone growth relies on the continuous cell division of chondrocytes in the proliferative zone and bone accretion at the metaphyseal side (Fig 3).

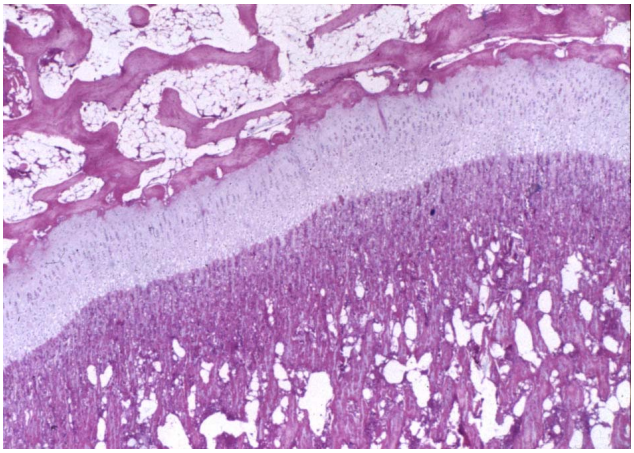

Fig 3. Microscopic section of the growth plate demonstrating endochondral bone formation. Courtesy of the Department of Pathobiology, Division Pathology, Faculty of Veterinary Medicine, Utrecht University.

3. Growth plate injuries.

Injuries of the growth plate can occur as long as the epiphyseal plate is not replaced by bone and usually occurs before he long bones have reached their full growth potential. Longitudinal bone growth in the dog ceases at approximately 10 months of age and is characterized by closure of the epiphyseal plates.[34] Growth plate fractures are classified according to the Salter-Harris system into 5 categories: type I, fracture through the growth plate; type II, fracture through the growth plate and metaphysis; type III, fracture through the growth plate and epiphysis; type IV, fracture through the growth plate, epiphysis and metaphysis, and type V, crush or compression injury of the growth plate.[21,145] The Salter-Harris system was designed to predict the prognosis of growth plate injuries. Based on clinical and experimental physeal fractures they postulated that most type I and type II fractures were restricted to the zone of hyperthrophic chondrocytes and thus should not

seriously affect longitudinal growth after careful reduction and stabilization. In dogs, the prognosis of these types of physeal fractures was proven to be less favorable. Disruption of the cells of the proliferative zone was found in the majority of traumatic growth plate injuries, which accounts for the high incidence of growth retardation in these patients.[59,89] In addition to decreased longitudinal growth, malformation of the limb is a common finding.

In dogs, growth deformity of the antebrachium is the most common limb malformation.[88] The antebrachium consists of the paired bones radius and ulna. Proximally these bones articulate with the humerus to form the elbow joint. The distal radius and ulna contribute to the antebrachiocarpal joint. During active growth the radius has a proximal and distal epiphyseal plate. The proximal growth plate contributes approximately 35%, whereas the distal plate contributes 75% to the total length of the radius.[34] The ulna has a proximal apophyseal growth plate, which accommodates for longitudinal growth of the olecranon. The ulna has only one distal epiphyseal growth plate, which is responsible for the entire longitudinal development of the ulnar diaphysis. This distal ulnar growth plate is shaped like an inverted cone to enlarge the proliferative zone area and thus the number of chondrocytes contributing to longitudinal growth. This adaptation is shape allows for synchronous longitudinal growth between radius and ulna. In short-legged dogs, this inverted cone shape is less obvious as growth rate in the radius and ulna is much slower.

By its high growth rate and configuration the distal ulnar growth plate is reported to be more vulnerable to trauma than the radial physes.[60,135] The typical presentation of growth retardation or premature closure of the distal ulnar growth plate is the radius curvus syndrome. An ulnar length deficit, cranial bowing of the radius, exorotation of the antebrachium, and valgus deviation of the distal limb in the carpus characterize this syndrome.[25,62,63] In the clinical situation, isolated disturbance of one physis is not common and usually both the distal radial and distal ulnar growth plates are involved.[128,140,183] The severity and localization of the growth disturbance within the radial and ulnar physes will vary, resulting in a heterogeneous presentation of the growth deformities. In addition to the growth deformity, asynchronous development of the radius and ulna can lead to incongruity of the elbow joint.[102,105,106,112] Asynchronous radial and ulnar growth can also result in carpal malalignment and subluxation.[61,117,122,134]

In summary, antebrachial growth deformities are characterized by a combination of length deficits, angular and rotational malalignment, elbow incongruity, and carpal subluxation. Osteoarthritis of the elbow and carpal joint is a common sequel of radial and ulnar growth deformities.

4. Healing of bone fractures

Bone fractures are characterized by discontinuity of bone architecture and loss of function. Bone healing is the reparative process by which bone regains its function. Two different mechanisms of bone repair can be distinguished, i.e., direct and indirect bone healing. Direct bone union is characterized by direct osteonal reconstruction, whereas indirect bone healing depends on the formation of an intermediate fibrous and cartilaginous callus.

Direct bone healing is achieved by internal remodeling of the Haversian systems without resorption of the fracture surfaces and intramembranous ossification. This type of healing is also known as contact healing and occurs with stable fixation and compression of the fracture surfaces. Under these conditions blood vessels can cross the fracture line followed by osteoclasts and osteoblasts, thus forming new Haversian systems. In small stable gaps of up to 0.2 mm in width direct union can also occur by direct deposition of lamellar bone. In larger stable gaps of up to 0.8 mm in width direct healing can proceed by the formation of cancellous bone.[41]

Indirect bone healing is characterized by the formation of an intermediate callus. This callus consists of fibrous and cartilaginous tissue. After bone fracture the sequence of events leading to bone healing can be described as hemorrhage in the fracture area, clot formation, inflammatory response, angiogenesis, proliferation of pluripotential mesenchymal cells, fibrous and cartilaginous callus formation, bone formation, and bone remodeling.[192] This sequence of events results in a gradual progressive stabilization of the fracture area with increasingly stronger and stiffer tissues. Callus formation can be subdivided on the basis of location into periosteal callus, intercortical callus, and medullary callus. The periphery of the callus consists of fibrous tissue, which encloses the more centrally located cartilaginous tissue of the callus. Bone formation in the callus proceeds from the periphery of the cartilaginous callus to the central area of the fracture zone. During this process of endochondral bone formation, cancellous bone is produced until the fracture gap is bridged. This cancellous bone is replaced by lamellar bone during remodeling, which can take up to several years. The extent of callus formation depends on several factors, including stability in the fracture area, age, and local blood supply. Increasing instability tends to result in a larger amount of callus formation. In contrast, excessive motion within the fracture zone will result in compromised angiogenesis and delayed union. Prolonged fracture union characterizes delayed union. Bone healing is progressive in delayed union and leads to full recovery of function. In nonunion, fracture healing stops altogether and results in either atrophic or hypertrophic nonunion. Atrophic or nonviable nonunion is characterized by resorption of the fracture ends without callus

formation. In hypertrophic or viable nonunion, callus formation is present at the ends of the fracture segments, but no bridging of the fracture gap occurs. Fractures can develop into nonunions by a variety of factors, including insufficient stability, inadequate reduction, interposition of soft tissues, compromised vascularity, and infection. In addition, systemic factors can contribute to the development of nonunion, including old age, hyperadrenocorticism, hypothyroidism, renal disease, osteoporosis, and GH deficiency.[75]

Fracture healing will proceed by a combination of direct and indirect bone union after osteosynthesis in the clinical situation. The cascade of events during fracture healing culminates in the recruitment of bone forming cells. Various angiogenic and osteogenic growth factors interact with pluripotential mesenchymal cells and their respective differentiated cell lineages during progression of fracture healing. These bone growth factors will be discussed in more detail in the next section of this introduction.

5. Distraction osteogenesis

Distraction osteogenesis is the formation of new bone under gradual mechanical distraction of two bone surfaces. Dr. Gavriil Abramovich Ilizarov was the first to develop distraction osteogenesis into a clinical treatment option, using a circular external skeletal fixation system.[84,85] The technique depends on a minimally invasive osteotomy of the bone while preserving soft tissues, periosteum, endosteum, bone marrow, and intramedullary blood vessels.[98] Stability of the external fixation is essential to allow new capillary blood vessels to bridge the osteotomy and allow for the lengthening procedure. After a latency period, the duration of which depends on the age of the patient and location of the osteotomy, gradual distraction is started with 1 mm per day, usually divided into 2 to 4 steps. New bone is formed in parallel columns extending from the osteotomy surfaces towards a central growth zone of the distraction gap. The growth zone that forms under the influence of tension-stress has features of both endochondral and intramembranous ossification.[5,8,9] After lengthening is ceased, the newly formed bone regenerate is allowed to mature and consolidate until the stage that the bone can support its physiologic load.

In humans, distraction osteogenesis has been used in treating a variety of skeletal conditions, including bone length deficits, growth deformities, bone loss after trauma or radical resection, and craniofacial surgery.[90,113,143,162] In dogs, distraction osteogenesis was introduced in the late 1980s mainly to treat growth deformities of the antebrachium and to a lesser extent of the crus.[105,106,116,117] In

addition to the clinical use of distraction osteogenesis, the dog has been used extensively as an experimental model.[4,8,56,57,95,100,118,129,155,186]

Distraction osteogenesis proved to be a successful model to study the role of growth factors during bone formation. Mechanical distraction stress induces the expression of transforming growth factor- β1 (TGF-β1), insulin-like growth factor-I (IGF-I), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) within the bone regenerate.[31,49,52,100,110,155,186,190] During distraction osteogenesis bone morphogenetic proteins-2 (BMP-2) and BMP-4 are produced by osteoblasts.[53,147] Recent interest has focused on angiogenesis of the distraction induced bone regenerate and the expression of vascular endothelial growth factor-A (VEGF-A), VEGF-D, angiopoietin-1 and angiopoietin-2.[28,44,51,77,82,121,130,142] These studies demonstrate the interdependence of the mechanical environment, angiogenesis, and bone formation during distraction osteogenesis. Expression of angiogenic genes and a proper mechanical environment are essential in supporting new vasculature for bone regeneration. Although our knowledge in this field is expanding, the complex cascade of growth factors regulating bone formation during distraction osteogenesis is still unclear.

6. Circular external skeletal fixation

External skeletal fixation systems have been used already for fracture stabilization from the mid-19th century onwards.[185] The concept of external fixation relies on fixation of bone by percutaneous pins linked with external connectors. External skeletal fixation systems can be used in various configurations. The three basic frame designs are the type I or unilateral configuration, the type II or bilateral fixation with transosseus pins, and the type III external fixation which combines types I and II to create a three dimensional frame.

Circular external skeletal fixation stands apart from the traditional external fixation systems as it is characterized by the use of metal rings surrounding the limb. Although circular external skeletal fixation systems were described in the early 20th century, Dr. Gavriil Abramovich Ilizarov was the first to develop this method of fixation into a clinical treatment modality.[84,85] Circular external skeletal fixation relies on the use of transosseus wires under tension rather than pins to connect the bone to the external fixation rings (Fig 4). The system is highly versatile and permits the use of partial rings and posts for transcutaneous pins thus encompassing the features of traditional external fixators. In addition, hybridization of the circular fixation with other external fixation configurations is possible. Initially, Dr. Ilizarov developed his circular fixation system to treat fractures and nonunion. In order to stabilize bone fractures under compression, threaded

connecting rods were supplemented to the system. Anecdotal, Dr. Ilizarov recognized new bone formation in a patient who accidentally distracted rather than compressed the fracture gap. This observation was the start of his pursuit culminating in the concept of distraction osteogenesis.

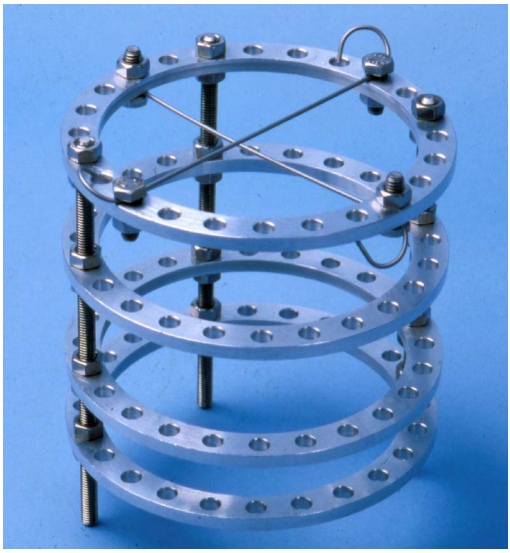

Fig 4. Circular external skeletal fixation system including rings, threaded connecting rods, and transosseus wires

Circular external skeletal fixation is used in veterinary medicine to treat a variety of fracture types.[105,106,108,115] Its use in companion animals is restricted mainly to fractures distal of the elbow and stifle joint. Although hybrid frame designs can be used on the humerus and femur, the use of transosseus wires proximal of the elbow and knee has high morbidity associated with the large muscle volume in these areas. In addition to fracture treatment, circular fixation has been used effectively to perform panarthrodesis of carpal and tarsal joints. The stability of the frame depends on several factors. The most important parameter is ring diameter.[107] Larger ring diameters require longer transosseus wires, which result in larger moments on the wire-bone and wire-bolt interface. Ring diameter is determined depending on the diameter of the limb adding approximately 2 cm on either side to allow for soft tissue swelling. Larger diameters will result in increased instability at the fracture site. The second most important parameter is wire tension.[7,107] Tensioning the transosseus wires will increase frame stability. Tension depends on the body weight of the patient and is applied by use of a dynamometric wire tensioner. An equivalent of 20 kg (±200 N) is used for dogs up

to 10 kg in body weight, an equivalent of 40 kg (±400 N) for dogs between 10 and 20 kg, an equivalent of 60 kg (±600 N) for dogs between 20 and 30 kg, and an equivalent of 80 kg (±800 N) for dogs 30 and 40 kg in body weight. Equivalents of up to 120 kg (±1200 N) can be applied in giant breed dogs.[84,85,105,106] In the basic configuration of the circular frame two transosseus wires are used per ring. The divergence angle between the transosseus wires has little impact on axial stability and is usually maintained between 60-90 degrees. Angles of divergence smaller than 60 degrees will decrease rotational and bending stability. Slippage of wires at the wire-bolt interface is a common cause for loss of initial stability. It is essential to tighten the nuts correctly after tensioning and to retighten them after cutting excess wire length and bending of the protruding wire ends to prevent auto-mutilation.

The basic design of circular fixators includes two full rings proximal of the fracture area each with two tensioned transosseus wires and two full rings distally. The rings are connected with 3 or 4 threaded rods to create a very stable frame. Transosseus wires with a diameter ranging from 1.2 to 1.6 mm are typically used in companion animals. Circular external skeletal fixation is very resistant to strain in bending and torsion. In contrast, it allows for micro-motion in the axial direction. This dynamic feature of circular fixation is considered to stimulate bone formation and thus bone healing.[6,8]

By analogy with all other external skeletal fixation systems, infection at the transosseus wire-skin interface is the most common complication.[105,106,113] This complication usually occurs at sites where there are large soft tissue masses covering the bone. Movement of soft tissues especially muscles will result in sliding movement at the wire-skin interface thus creating an easy access for bacteria. Avoiding large muscle masses can reduce this complication to a minimum. Proper care of the circular external skeletal fixation system especially of the transosseus wires remains essential as wire-tract infection is painful and restricts function. Bandages are effective in stabilizing the soft tissues adjacent to the transosseus wires and in protecting the skin-wire interface. Wire-tract infection should be treated aggressively with antibiotics and local wound care to avoid progression of the infection to the transosseus wire-bone interface. Established infection will necessitate transosseus wire removal and revision of the frame design.

Circular external skeletal fixation is well tolerated with proper care. Dogs are able to bear weight shortly after surgery, which is beneficial for both bone healing and musculoskeletal function. Dogs can be managed on an outpatient basis with weekly checkups. Exercise should be limited to leash walks until bone has consolidated and the frame can be removed. Although circular external skeletal

fixation is a valuable asset in treating fractures, its strength lies in the management of growth deformities and bone deficits.[47,116,117]

7. Hormonal regulation of bone formation

The endocrine regulation of bone is mediated through several hormones, including growth hormone (GH), calcitonin, parathyroid hormone (PTH), calcitriol, androgen, and estrogen.[126,167,174] Growth hormone is essential for longitudinal growth occurring in the epiphyseal plate through the process of endochondral ossification.[12,33,54,125] Part of the actions of GH is mediated through the induction of IGF-I, but GH also directly promotes longitudinal bone growth.[158,188] The expression of growth hormone receptor (GHR) within the growth plate is consistent with this finding.[37,71,93] At least in humans, GH has a major role in the maintenance of bone mass in the adult skeleton by regulating bone remodeling through a complex interaction of circulating GH, insulin-like growth factors (IGFs), insulin-like growth factors binding proteins (IGFBPs), and locally produced IGFs and IGFBPs.[177] Relative or absolute GH deficiency thus results in osteoporosis.[18,146,159] In dogs, GH deficiency or estrogen depletion does not lead to osteoporosis.[176] Osteoporosis does occur in renal or alimentary induced hyperparathyroidism in dogs.[157]

Growth hormone has been used successfully in treating GH-deficiency related osteoporosis and postmenopausal osteoporosis in humans.[101,146] In view of its role in bone metabolism, GH has been used experimentally to stimulate osteogenesis. Systemic GH application effectively stimulated bone formation in fracture models in different species, including dogs.[10,149,191] In addition, GH was demonstrated to enhance consolidation of the bone regenerate after distraction osteogenesis.[11,136]

Calcitonin, PTH and calcitriol are crucial in maintaining calcium homeostasis.[123,166,174] Calcitonin is synthesized by the clear cells of the thyroid gland and stimulates calcium deposition while inhibiting bone resorption. Parathyroid hormone is synthesized by the parathyroid glands, which are located in or adjacent to the thyroid glands. The function of PTH is to mobilize calcium from bone and reabsorb calcium in the kidney. Continuously increased PTH levels increase osteoclast activity mediated through the shrinkage of osteoblasts thus exposing bone to osteoclasts, whereas intermittent treatment with PTH stimulates osteoblast activity and thus bone formation.[67]

Skeletal growth and puberty are connected since sex hormones estrogen and androgen play a role in cartilage growth and endochondral ossification. Early castration results in delayed closure of the growth plate with subsequent increased

final bone length.[144] In adult dogs, estrogen and androgen do not seem to be essential in maintaining bone mass. Ovariectomy or orchydectomy, even at a young age, does not result in clinical osteoporosis. Nevertheless, estrogen depletion after ovariectomy does negatively affect cancellous bone growth and increases cortical porosity.[156,193]

8. Skeletal growth factors

The family of insulin-like growth factors (IGF) consists of IGF-I, IGF-II and insulin. IGF-I and IGF-II are major constituents of both local and systemic growth factors.[125,196] There are three receptors each with the highest affinity for their specific IGF. These receptors, particularly for IGF-I, are present in almost all tissues. IGF-I is mainly produced in the liver, but also in peripheral tissues including bone.[46,158] Knowledge about the role of IGF-II in bone formation and bone healing remains limited.[13] IGF-I and IGF-II are important as local growth factors for osteoblast survival and apoptosis.[81] By modulating osteoblast-osteoclast interactions IGF-I and IGF-II are critical in bone remodeling and sustaining bone mass.[78,80] In vitro, IGF-I stimulates existing bone resorption by existing osteoclasts and the formation of new osteoclasts from precursor cells.[119] Upregulation of IGF-I and IGF-II expression occurs during bone healing.[164] IGF-I is also expressed in the bone regenerate during distraction osteogenesis.[170] Local treatment with IGF-I stimulates bone healing and bone consolidation after distraction osteogenesis.[97,165]

Six high-affinity IGF binding proteins (IGFBP) regulate the actions of IGF-I and IGF-II.[92,120] The IGFBPs are produced by osteoblast and IGFBP-4 and IGFBP-5 are the most abundant.[74,120] In general, IGFBP-1, -2, -4, and -6 inhibit and IGFBP-3 and -5 stimulate osteoblast function.[30,43,50] Overexpression of IGFBP-2 was demonstrated to impair long bone development in vivo by blocking the ability of IGF-I and IGF-II to promote cell proliferation and matrix synthesis.[58] Growth hormone treatment in postmenopausal women resulted in elevated serum levels of IGF-I, IGF-II, IGFBP-3, IGFBP-4, and decreased serum levels of IGFBP-1 and IGFBP-2. Serum levels of IGFBP-4 and IGFBP-5 correlated with bone mineral density in GH-deficient adults. Growth hormone replacement therapy increased both IGFBPs.[172]

Although IGFs and IGFBPs are important for bone maintenance and formation their precise role is still to be elucidated.

The transforming growth factor-β superfamily consists of the transforming growth factor-βs (TGF-βs) and the bone morphogenetic proteins (BMPs). The TGF-βs are multifunctional peptides expressed in mammals as highly homologous isoforms, TGF-β1, TGF-β2, and TGF-β3. The TGF-βs act via autocrine and

paracrine modes to control a variety of developmental processes.[14] TGF-βs stimulate osteoblast proliferation and matrix production. In this way they are important regulators of bone formation and fracture repair[17,19,163]An early decline in serum TGF-β1 levels was demonstrated in patients with delayed fracture healing.[197] TGF-βs have been used successfully to stimulate fracture healing. [16] In distraction osteogenesis, TGF-βs are typically expressed during the phase of active lengthening.[110,170] Experimental stimulation of bone consolidation with TGF-β1 during distraction osteogenesis was not successful indicating a role for TGF-βs especially in the early stages of osteogenesis.[138,151]

BMPs were first described as factors capable of inducing new bone.[179]The group of BMPs comprises at least 15 growth factors, which are highly osteoinductive.[29] Besides their role in osteogenesis, BMPs exert essential functions during embryogenesis.[184] BMPs bind to two distinct types of transmembrane receptors with serine-threonine kinase activity. The activated receptors phosphorylate Smad proteins, which act as intracellular signal mediators.[24,104] These Smads regulate the expression of target genes. The TGF-βs signal through the same Smad pathways. The BMPs can induce endochondral bone formation through the proliferation and differentiation of chondrocytes and osteoblasts.[175,187] BMP-2, BMP-4, and BMP-7 expression was highest during the early phases of fracture healing.[161] The expression of these BMPs diminished after the initial phases of osteogenesis. By analogy, BMP-2, BMP-4, and BMP-7 expression was most prominent during the phase of active lengthening during distraction osteogenesis, to decrease during the phase of bone consolidation.[137] BMP-2 and BMP-7 are the most potent osteoinductive growth factors and have been used both experimentally and clinically to stimulate bone formation.[73,91,181] Part of the mitogenic action of BMP-7 is mediated through the modulation of IGF-II secretion and the balance between stimulatory and inhibitory IGFBPs.[96] BMP-2 was most effective in stimulating bone healing in an experimental fracture model when administered immediately postoperatively.[124] The delivery systems by which BMPs are administered also play an important role in their effectiveness.[152] In dogs, BMPs have been used successfully to treat nonunions and segmental bone defects.[35,79]

The group of fibroblast growth factors (FGFs) consists of 18 growth factors. Fibroblast growth factor-1 (FGF-1 or acidic FGF) and fibroblast growth factor-2 (FGF-2 or basic FGF) are the prototypic members of the FGF family. Fibroblast growth factors and their receptors have an important role in the control of endochondral and intramembranous bone formation.[14,72] The expression of FGFs is closely related to the expression of vascular endothelial growth factors (VEGFs). Both FGF-2 and VEGFs are expressed in the early stages of distraction osteogenesis and are considered essential for angiogenesis.[82,130,195] Expression of

VEGFs occurs at the osteogenic front during distraction osteogenesis and precedes the expression of BMPs by osteoblasts. This demonstrates that angiogenesis is induced before osteogenesis.[160] VEGF was capable of stimulating bone healing in an atrophic nonunion model.[45] In contrast, VEGF application was not effective in increasing bone consolidation in a distraction osteogenesis model.[44] Combined delivery of angiogenic (VEGF) and osteogenic (BMP) factors was more effective in enhancing new bone formation than application of the single factors.[83]

Platelet-derived growth factor (PDGF) was initially isolated from platelets, but was found to be expressed by skeletal and a variety of non-skeletal tissues.[19] Nevertheless, platelets are the major source of this growth factor, which is released following platelet aggregation. Aggregation of platelets typically occurs after fracture and soft tissue trauma. This emphasizes the role of PDGF in fracture repair. Platelet-derived growth factor increases the replication of cells of osteoblastic and osteoclastic lineages.[14]

Hepatocyte growth factor (HGF) was discovered as a potent growth-promoting agent in liver cells. HGF is expressed in most tissues and plays an important role in tissue repair. This growth factor stimulates both osteoclasts and osteoblasts, thus regulating bone remodeling. In addition, HGF is expressed at the fracture site and induces upregulation of BMP receptors.[86] In this way, HGF facilitates BMP signaling and stimulates bone healing.

The identification of the receptor activator of nuclear factor kappaB ligand (RANKL), its cognate receptor RANK, and its decoy receptor osteoprotegerin (OPG) resulted in a new perspective on osteoclast function and bone homeostasis.[169] Osteoclast precursors express RANK and differentiate into osteoclasts after recognizing RANKL through cell-to-cell interaction with osteoblasts and stromal cells.[166,171] Membrane bound colony-stimulating factor-1 (mCSF-1) plays an important role in osteoblast-mediated osteoclastogenesis.[189,194] Osteoprotegerin acts as a soluble receptor antagonist for RANKL that prevents it from binding to and activating RANK. Hepatitis C-associated osteosclerosis is characterized by diffuse osteosclerosis, decreased osteoclast numbers, and increased OPG serum levels in adult humans. An imbalance in the OPG/RANKL system is supposed to be responsible for these findings.[114] There is increasing evidence that the OPG/RANKL system links the skeletal with the vascular system.[141,148] These findings may lead to new therapeutic regimes in preventing bone resorption. For instance, GH replacement therapy increased OPG levels, which may lead to a positive bone balance by inhibiting osteoclastogenesis.[103,178]

Ghrelin, the endogenous ligand for the GH secretagogue receptor (GHS-R) is a recently discovered brain-gut peptide involved in GH secretion and energy homeostasis.[66] In addition, ghrelin is widely expressed in several tissues, where it might therefore act as a paracrine or autocrine factor. Ghrelin directly regulates

bone formation by stimulating osteoblast proliferation, differentiation, and function and by inhibiting apoptosis.[66,94,111] Ghrelin is also synthesized and secreted by chondrocytes.[23] In future, ghrelin and synthetic GHS-R ligands may be effective in modulating bone homeostasis.

9. Bone markers

Bone markers are used to monitor bone metabolism non-invasively. These markers can be divided into markers of bone formation and markers of bone resorption, respectively. Although the commercially available bone marker assays were designed initially for use in humans, several of these assays are also validated to monitor bone metabolism in laboratory animals, dogs and horses.[20,26,27,99]

The markers of bone metabolism can be subdivided into enzymatic markers and metabolic products of bone formation and resorption, respectively.[22,153,154] Enzymatic markers include bone-specific alkaline phosphatase (BAP) and tartrate-resistant acid phosphatase (TRAP)[90,127]. Bone-specific alkaline phosphatase is an osteoblast-related marker of bone formation.[38,69] Tartrate-resistant acid phosphatase-5b (TRAP-5b) is an osteoclast-related marker of bone resorption. [76,173] Serum or plasma markers of metabolic products of bone metabolism include osteocalcin (OC), carboxyterminal propeptide of type-I procollagen (PICP), aminoterminal propeptide of type-I procollagen (PINP), cross-linked carboxyterminal telopeptide of type-I collagen (ICTP), and C-terminal cross-linked telopeptide of type-I collagen (CTX). Osteocalcin is an osteoblast-related marker of bone formation, but its precise function is unknown.[168]. Increased bone formation was present in OC-deficient mice, indicating a suppressive role of OC on bone formation, possibly through inhibition of osteopontin.[42] Markers PICP and PINP are metabolic products of type-I collagen synthesis and hence bone formation.[36,133,167] Markers ICTP and CTX are products of type-I collagen breakdown and hence bone resorption.[2,20,132,150] Marker ICTP is released through the actions of matrix metalloproteinases (MMPs) and is therefore also known as CTX-MMP. Marker CTX is formed from collagen I by cysteine proteinases, including cathepsin K.[70] The collagen type I C-telopeptide is susceptible to molecular rearrangement. In newly synthesized collagen this site is in the native form, but during aging a spontaneous reaction occurs, resulting in three age-modified isomerized and racemized fragments. These modified forms of CTX may provide new diagnostic and monitoring tools in evaluating bone disease.[32]

Bone markers have been used successfully in humans to monitor metabolic bone disease and the effect of treatment. In rheumatoid arthritis, characterized by increased bone resorption, ICTP levels were elevated while OC levels were

decreased.[1] Growth hormone treatment in GH-deficient patients resulted in transient changes in BAP, OC, and ICTP levels.[18,146,159] Serum BAP and PINP were the most sensitive markers for monitoring treatment efficacy in Paget's disease.[3] In Cushing's syndrome, the markers OC and CTX decreased consistent with increased bone resorption.[36] The markers BAP, OC, PINP, and CTX were most effective in monitoring osteoporosis.[39,40] Although bone mass measurements at the present time still supply most information about fracture risk, markers of bone resorption may be useful in predicting fracture risk in osteoporosis.[68] In chronic renal failure, PICP and ICTP have been used as markers of bone formation and resorption, respectively.[133] Bone markers have also been used to monitor bone growth. In pubertal boys and girls, markers of bone metabolism related positively to growth velocity.[182] This finding could be of great interest in monitoring the rapid growth phase in dogs.

In dogs, normal values for BAP, OC and ICTP have been established and are not affected by breed size.[20,99] By analogy with humans, bone markers demonstrate a circadian rhythm in the dog.[99,109,131] Therefore, sampling should be standardized both in an experimental and clinical setting. Bone markers have not been used extensively to monitor bone pathology in dogs. Experimentally induced osteomyelitis increased ICTP serum levels.[132] Orchydectomy in male beagle dogs resulted in decreased BAP and OC levels indicating an imbalance in bone metabolism with diminished bone formation.[65] In canine appendicular osteosarcoma, BAP proved to be a prognostic factor in predicting survival time.[48] Experimentally, OC was used effectively to monitor new bone formation following distraction osteogenesis.[15,55,100,155] Although bone markers can be an adjunct in monitoring bone metabolism, they cannot replace bone histomorphometry in orthopaedic research at the present time.

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