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Title page

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Running title: **The periosteum**

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The periosteum – Part 1: Anatomy, histology and molecular biology

Historical aspects

Since the time of Duhamel and John Hunter it has been the belief of anatomists and surgeons that the periosteum is osteogenic. In 1757 Duhamel and Monceau reflected the periosteum from the bone and fitted around it a silver ring, over which the periosteum was sewed. After a period of several months the ring was completely covered with bone and from this observation they concluded that the periosteum secreted bone.[35] In the mid 1800s, Dupuytren proposed that the cartilage of fracture callus originated from periosteum and bone marrow.[36] In 1867 Ollier proved that the deep cellular or osteogenic layer of a free periosteal graft is able to produce bone. This view was not disputed until in 1912 when Sir W. Macewan published his work *The Growth of Bone* in which he described many experiments which seemed to demonstrate that the periosteum cannot be considered osteogenic, and that it must be viewed merely as a limiting membrane of much the same nature as the sheath of a muscle or the capsule of one of the viscera. This observation of a periosteum as merely a limiting membrane was confirmd by the Gallie and Robertson in 1914.[44] Then Lacroix in 1945. demonstrated the osteogenic capabilitiy of mature periosteum.[65]

Anatomical considerations

The periosteum is specialized fibrous tissue in a form of fibro-vascular membrane. This well vascularized fibrous sheath, covers the external surface of most bones and is absent from articular surfaces, tendon insertions, or sesamoid bone surfaces.[60] The periosteum and bones are bound together by collagen fibers called Sharpey's fibers that penetrate into bone. The direction of collagen fibers is determined by tension forces (Fig. 1). These fibers penetrate entire cortex at the sites exposed to high amount of tension forces and the results are tight junctions of tendons and bones.[136] In the region of the diaphyses of long bones periosteum is thicker (2-3 mm) and easily separated from the underlying bone. It is strongly fused with bones in metaphyseal and epiphyseal region where it is thinner.

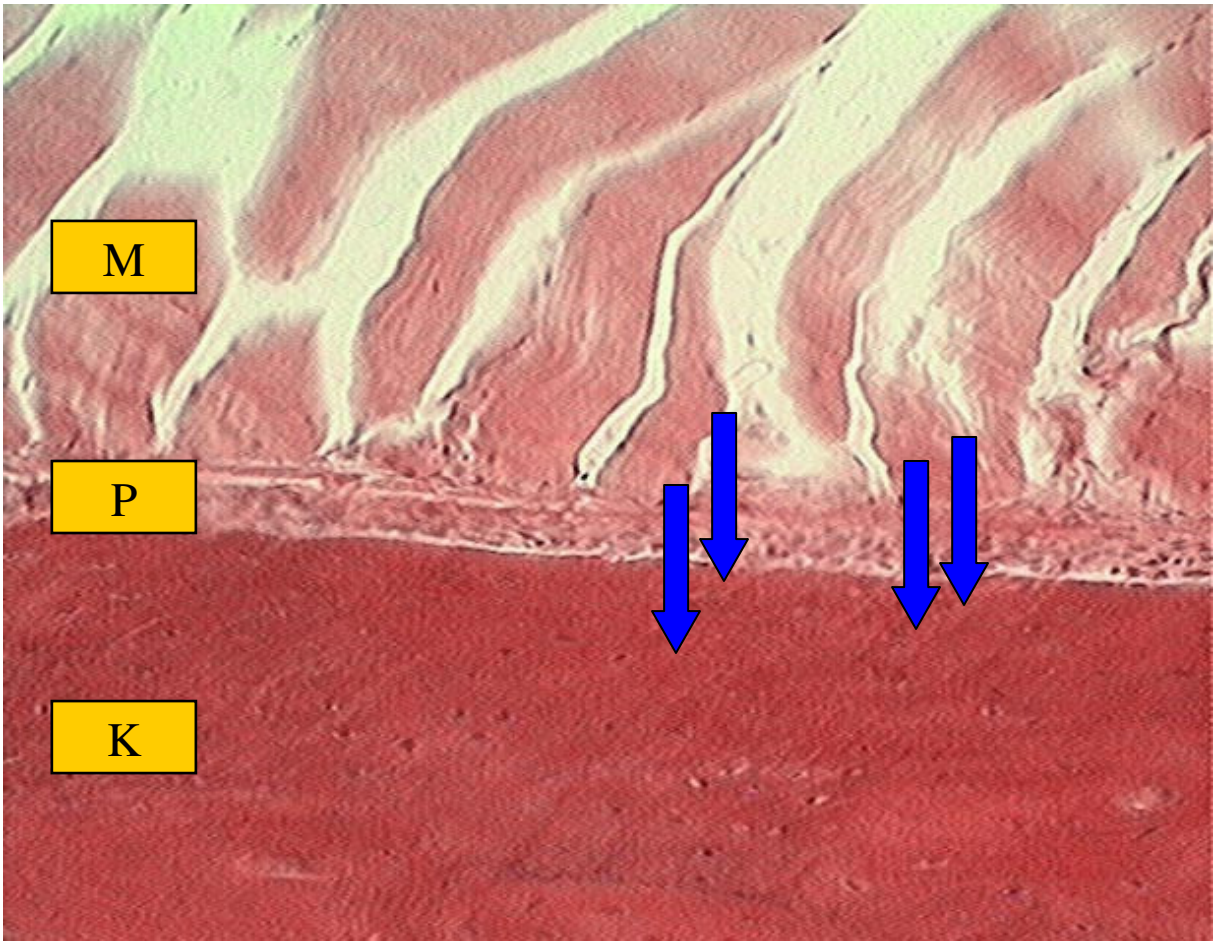

Figure 1 Cortex (K), periosteum (P) and muscle (M). Collagen fibers (*Sharpey's fibers*, blue arrows) penetrate from periosteum to bone matrix.

The main feature of children's bone is to grow, wrapped with elastic, firm periosteum. This explains why children fractures have some specific biomechanical features: bone fractures without the disruption of the periosteum (subperiostal fractures) or intact periosteum of the concave side of the fracture (greenstick fracture).[59] With growth, the periosteum becomes thinner and loses elasticity and firmness.[91] It is especially compliant on tensile forces and tearing which results in the disruption of the periosteum in the level of bone fracture in adults. The periosteum is highly vascularized and innervated and contains large amounts of lymphatic vessels.[53] It contains different types of nerves: sensory and vasomotor nerves. These vasomotor nerves regulate vessel tone by regulation of precapillary sphincters and capillary blood flow. Pain fibers with nociceptors are highly expressed which explains intense pain that follow periosteal injuries.[74]

Microscopic features

Generally, periosteum is composed of outer fibrous and inner cellular layers and does not supply epithelial cells, though periosteum has the potential to produce collagen.[25] The structure of the periosteum in terms of ultrastructure and functional organization was not definitively understood until recently. The original division in two anatomical layers was made by Tonna in 1965, and only in 1986 Tang and Chai clearly delineated osteogenic cells of cambium from fibroblasts (fibrous layer).[124,128]

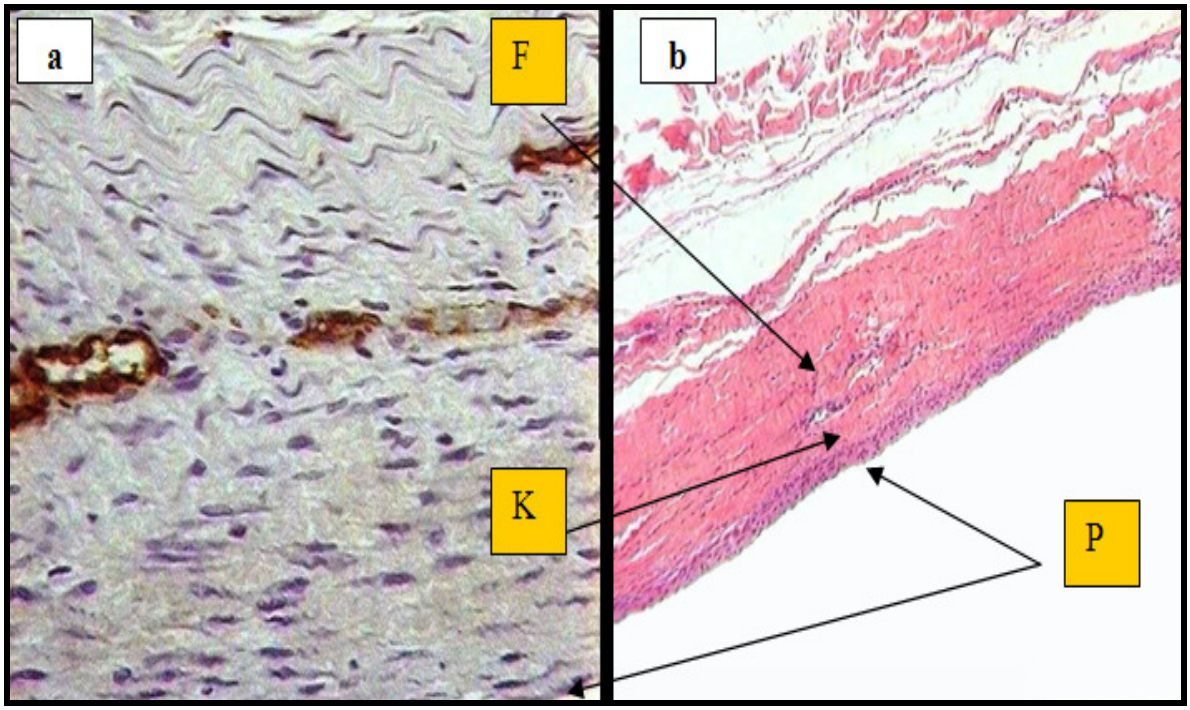

Figure 2 The periosteum of sheep tibia. a) Magnification ×250, b) magnification ×25. Photomicrograph of normal periosteum attaching to bone. Periosteum consists of two clearly divided layers: osteogenic, cambium (K) and fibrous (F) layer. Periosteal surface (P) adjacent to the cortex.

Microscopically (Fig. 2), the periosteum consists of outer, fibrous, firm layer (collagen and reticular fibers) and inner, proliferative layer (cambium) which lies adjacent to bone and contains osteoblast and osteoprogenitor cells (Fig. 3). Cambium is capable of: a) forming normal lamellar bone apposition on cortical bone that grows in width and b) forming primary, woven bone after fracture.[54,103,124,129] The outer fibrous layer provides elasticity and flexibility, whereas the inner cambium is the osteogenic and contains 3 or 4 cell layers, including osteoblasts and preosteoblastic cells.[24,27,119]

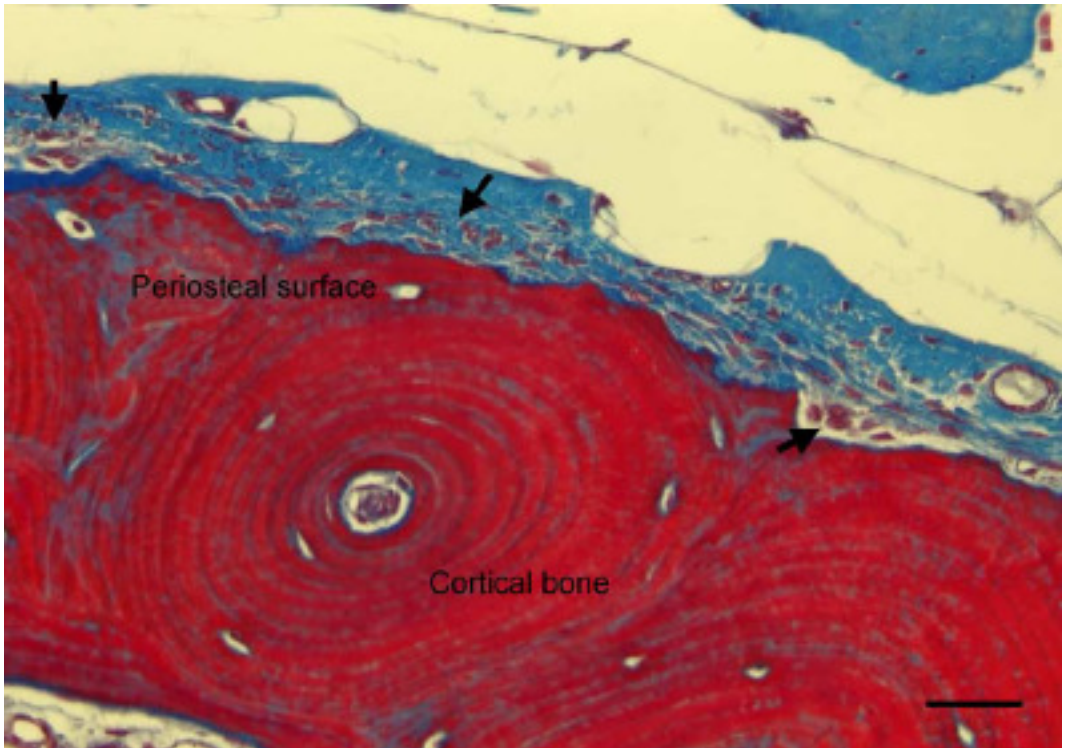

Figure 3 Periosteal covering of the human femoral midshaft. Note the abundance of cells (arrowheads) near the periosteal surface comprising the cambium layer stained with Masson trichrome. Magnification ×400, bar = 25 μm.
From Allen MR, Hock JM, Burr DB. Periosteum: biology, regulation, and response to osteoporosis therapies. Bone 2004;35:1003-12 Copyright Elsevier.

The first division of the periosteum into three layers was made by Squier et al.[119] in 1990 with the analysis of periosteal morphology of the dog with light and electron microscope.

Zone I consists mainly of osteoblasts arranged in the layer adjacent to the bone surface in a form of simple epithelium and a supraosteoblast layer of smaller, compact cells.[6] Adjacent to primary (immature) bone, during intense synthesis of extracellular matrix, osteoblasts are cuboidal, arranged as stratified epithelium, with basophilic cytoplasm with high levels of alkaline phosphatase (Fig. 4).[38] With the decrease of activity, osteoblasts elongate and basophilic characteristics of the cytoplasm decrease. Layer over the osteoblasts consists of small, spindle cells with scarce endoplasmic reticulum that are similar to fibroblasts. These are osteogenic progenitor cells which differentiate into osteoblasts. Fibrous tissue consists mainly of collagen and small amount of elastic fibers.[125] Fibroblasts are scarce and blood vessels are almost completely lacking.[114] This is the thinnest part of the periosteum (also called germinative layer).

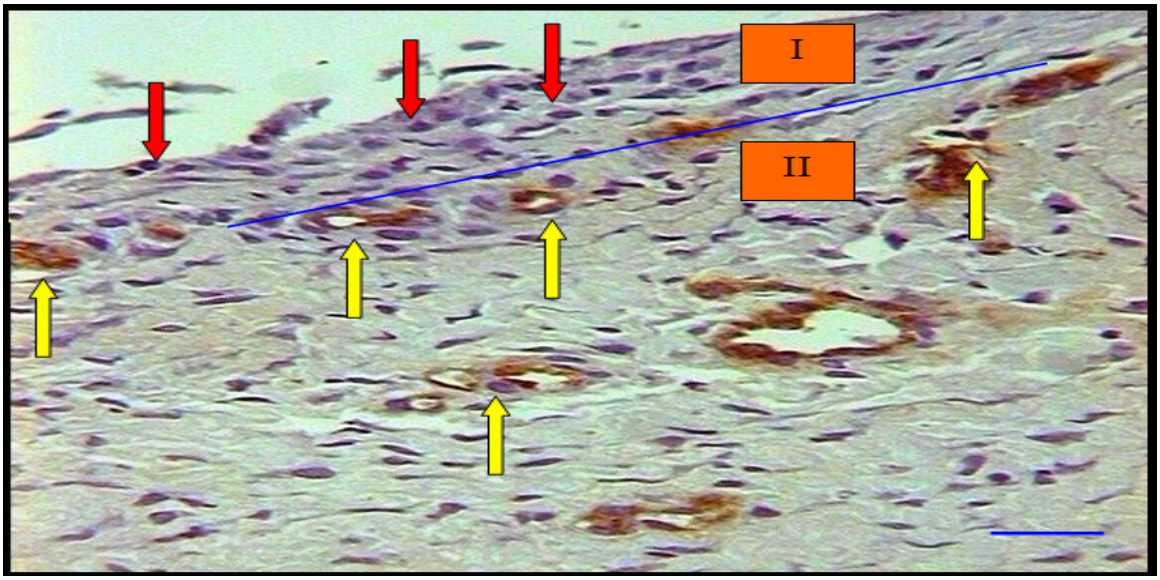

Figure 4 Periosteum of the sheep tibia. Zone I: basophilic osteoprogenitor cells (red arrows) of germinative layer in transition to Zone II (blue line). Zone II: transparent zone with capillaries (yellow arrows) consists of extracellular matrix and fibroblasts. Magnification ×25, bar = 15 μm. Hemalaun-eosin. Imunohistochemical staining with CD 31 and CD 34 (von Willenbrand factor).

Zone II is relatively transparent zone with capillaries and amorphous extracellular matrix making the most voluminous part (Figs. 5,6). The fibroblasts constitute the most of the cellular component and collagen fibers are abundant and both structures occupy one quarter of this layer. Fibroblasts are properly arranged in thin bunches, thinner than in other layers of the periosteum.[119]

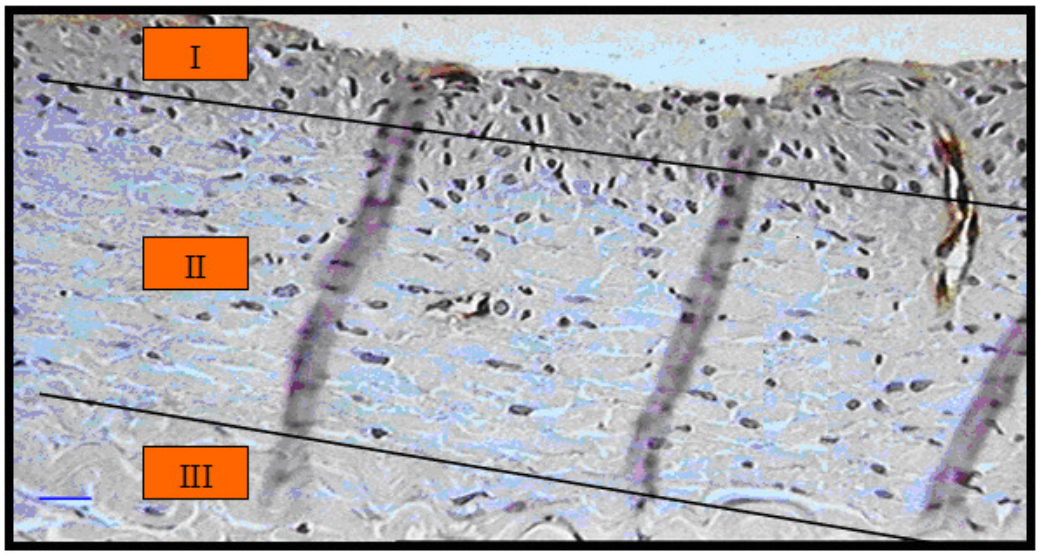

Figure 5 Periosteal division into three zones. Zone II (transparent zone) consists of extracellular matrix and fibrolasts. Magnification ×25, bar = 15 μm. Hemalaun-eosin. Imunohistochemical staining with CD 31 and CD 34 (von Willenbrand factor).

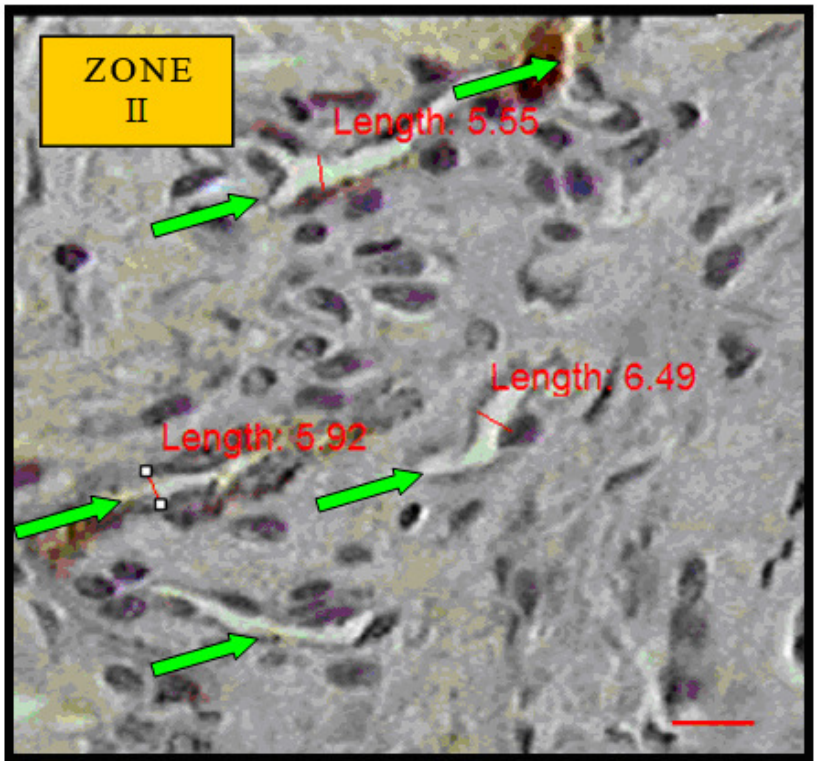

Figure 6 Zone II of the sheep tibial periosteum. Zone II with capillaries (green arrows). Capillary diameter is 5.55-6.49 µm. Magnification ×250, bar = 15 µm. Hemalaun-eosin. Imunohistochemical staining with CD 31 and CD 34 (von Willenbrand factor).

Blood vessels are numerous in this layer, mostly capillaries (Figs. 4,6). Together with dense capillary network this layer contains abundance of endothelial pericytes.[32] Pericytes are polymorphic cells of mesenchymal origin, which contain multiple, branching cytoplasmic processes that partially surround capillaries. Pericytes are found in the microvasculature of connective tissue, nervous tissue, muscle tissue and the lungs.[115] These cells have ability to contract and hence may regulate blood flow in the microvasculature.[23] Pericytes may also function as resting stem cells and differentiate into smooth muscle cells.[81] They may also play a regulatory role in controlling capillary proliferation during wound healing,[31] and support capillaries in maintaining structural rigidity of the micro-vessel wall.[29] Pericytes are cells in physical contact with capillary endothelial cells, with the ability to differentiate into numerous cell types, including osteoblasts.[14,101,133] These cells may serve as a supplementary source of osteoprogenitor cells[32] and may be more important in periosteal bone formation due to their greater abundance in periosteum[20] than in endosteal bone surface apposition.[14] Cultured pericytes mineralize *in vitro* and synthesize the osteoblast marker, alkaline phosphatase, as well as bone matrix proteins, including osteocalcin,[20] osteonectin, osteopontin, and bone sialoprotein. These cells form an osteogenic tissue that mimics bone-derived tissue, both spatially and temporally,[38] and responds to osteogenic stimuli, such as BMP and parathyroid hormone.[101]Sympathetic nervous fibers in this layer are much denser than in the bone.[74] Extracellular matrix and fibroblasts are less susceptible to histologic staining and this layer of the periosteum is less salient and is brighter, and together with zone I is called cambium (from Latin, meaning *to exchange*). The only protein that is present in the higher amount in periosteum than in the bone is periostin.[55,72] Predominantly it is located in the preosteoblasts which secrete periostin in the extracellular matrix. The original term for this protein is OSF-2,

and the highest concentration is found in the disrupted periosteum. The synthesis of periostin is increased 4-fold during the first three days after the fracture.[72] The concentration decreases with the progression of differentiation of osteogenic progenitor cells and the activity of osteoblasts. Still the synthesis and the role of the periostin are unclear. It seems that it is responsible for the interaction of cells and extracellular matrix, as a mediator, during mechanical changes in the periosteum. Also its role is probably in the osteoblast differentiation.[55,90]

Zone III consists of numerous fibroblasts with collagen fibers in scarce extracellular matrix (Fig. 5). The blood vessels are scarce, mostly capillaries. This zone is easily perceivable because of high amount of collagen fibers and their susceptibility for histologic staining. The most important characteristics of collagen are firmness, inextensibility and insolubility. Collagen fibers make network of thin fibers in ambiguous directions.[103,119] This layer is called 'fibrous layer' of periosteum. The zone I is thin in contrast to zones II and III which are several fold thicker. These significant quantitative differences in the periosteal structure in all three zones are constant despite of the region and the location on the bone and indicate persistent periosteal microanatomy.[4,72, 119]Today, it is clear that morphology of the periosteum depends not only upon the species but also upon the age. Periosteal fibroblast number and fibrous layer thickness decrease with age,[127] although atrophy of the fibrous layer is less than that of the cambium layer.[91] Vessel density throughout the periosteum also declines with age but retains the capacity to increase when activated by mechanical loading or fracture repair.[38] These age-induced changes may help explain why periosteal cells from older subjects fail to form mineralized nodules in culture,[85] and why periosteal bone formation rate[40] and responsiveness to hormones and cytokines[95] decline with age. During aging the size and the number of the cells decrease while the size and the thickness of the collagen fibers increase.[28] Cellular density of the cambium layer is 3-fold higher than the fibrous layer but the ratio is constant and is not changed with aging. Absolute and relative values of total periosteum thickness and the thickness of each layer are decreased.[4,72,91] The main feature of the morphologic changes of cambium layer during aging is almost dramatical decrease[127]and elongation[38] of osteoblasts. This reduction in osteoblast number may contribute to the apparent atrophy and thinning of the cambium layer that occurs with age.[91] Periosteal fibroblast number and fibrous layer thickness also decrease with age,[127] although atrophy of the fibrous layer is less than that of the cambium layer.[38,91] This, biologically impaired and reduced periosteum has small reparatory potential with slower response rate on stimulation with cytokines and hormones (longer fracture healing time). Periosteal expansion occurs throughout life. The rate of expansion is high during puberty,[17] slower during the adult years[106,117] and in women, accelerated again after menopause.[1] Independently of other changes, expansion of the periosteal surface increases the strength of long bones and decreases the risk of fracture.[89]

Site-specific differences in periosteal anatomy or activity clearly exist throughout the skeleton. It is well know that the calvarial periosteum is uniquely regulated compared to the axial skeleton, and that cellular periosteum is scarce at the femoral neck.[98] The existence of periosteum at the femoral neck is commonly debated. Early observational[92,96] and histological[10] studies suggest that human femoral neck lacks a periosteum. The absence of callus formation following femoral neck fractures in adults supports these observations.[37,41,60,92,122]. Despite these studies there are some opposed observations claiming that the femoral neck periosteal covering exists.[8,34,98,112] Periosteal cellularity at the femoral neck is significantly lower than in the diaphyseal region even in young adults. Twenty percent of the femoral neck surface has cellular periosteum which suggests that anabolic osteogenic

therapies may be effective in strengthening this clinically relevant site. Periosteal cells have greater sensitivity to mechanical[61] and pharmacological[82] stimuli compared to marrow cells and even limited cellular periosteum may be sufficient for enhancing periosteal apposition. These cells likely do serve to expand the periosteal diameter, as the femoral neck experiences age-associated radial expansion.[11,106,117] It may be, however, that limited quantity of cells limits the rate of expansion, resulting in less than optimal bone geometry and therefore elevated fracture risk. Alternatively, these data may present supporting evidence that the femoral neck exhibits an alternative means of periosteal apposition. Previous studies have documented that both periosteal calcification and calcified fibrocartilage undergo osteonal remodeling.[134,140] Although this study did not document any calcified fibrocartilage, the abundant periosteal mineralized tissue did contain individual osteons, clearly separated from the periosteal bone surface, in some regions. Such mechanism could be an alternative explanation for femoral neck periosteal expansion with age. Thus, rather than circumferential lamellae being laid down on the periosteal surface and subsequently remodeled into osteons, as occurs in diaphyseal bone, mineral accumulates separate from the periosteal surface with subsequent osteonal remodeling necessary for incorporation into the existing bone. The highly irregular surface of the femoral neck, as compared to the relatively smooth periosteal surface of diaphyseal bone, certainly supports this hypothesis although further study is necessary.[2]

There are few studies that specifically address the site-specific differences,[4,83,113] yet clear differences in periosteal bone formation rates exist among skeletal sites.
Because of ligament and tendon muscle attachments, and fibrocartilage, on some areas of the periosteal surface means that periosteal cells are exposed to the different physical environments in contrast to more frequently studied endosteal cells, which are bathed in hematopoietic marrow. Compared to endosteal osteoblasts, periosteal osteoblasts exhibit greater mechanosensitivity to strain,[61] a lower threshold of responsiveness to parathyroid hormone,[82] higher levels of expression of proteins such as periostin[55,90,123] and more estrogen α receptors.[21] These differences in threshold sensitivity to physical, hormonal, and mechanical stimuli may underlie the differences in periosteal and endosteal surface responses to therapy.[39]
Periosteum has cholinergic sympathetic innervation (Fig. 7). Adult periosteum contains VIP-immunoreactive fibers associated with periosteum, as well as catecholaminergic fibers associated with blood vessels.[52,53] VIPergic and cholinergic properties are present in the same fibers.[121] Tracing studies indicate that periosteal VIP-immunoreactive fibers of the ribs and sternum originate from thoracic sympathetic ganglia.[53]

Periosteal circulation

The arterial supply of the long bones consists of the nutritional arteries and of numerous vessels entering the bone from the periosteum.[5,51] The periostal circulation is an important part of bone vascularization. The blood supply of the periosteum is derived from four vascular systems.[116]

Intrinsic periosteal system

Intrinsic periosteal system is located between fibrous layer and cambium, mostly in zone II (Fig. 7).[116]

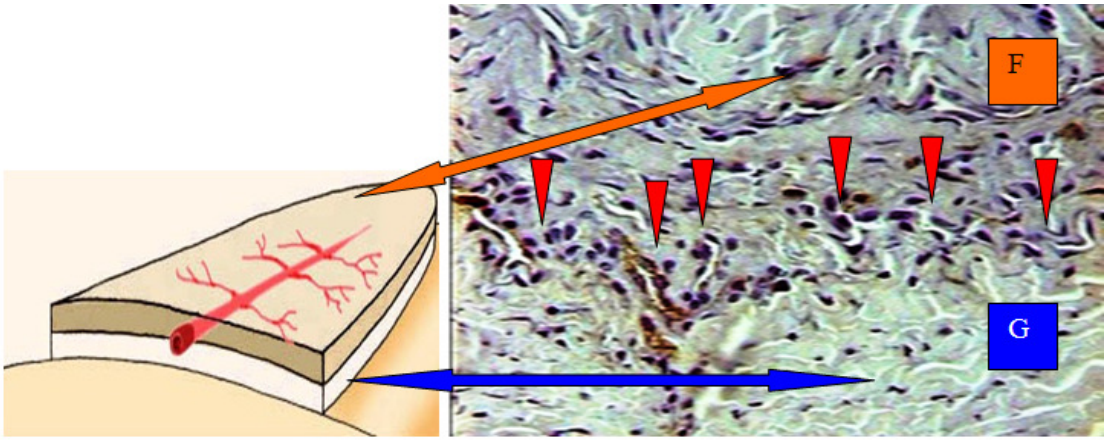

Figure 7 Location of intrinsic periosteal system between (G) germinative, cambium and (F) fibrous layer (red arrowheads).

These are terminal branches of nutritive periosteal system. These branches form a net of (a) longitudinal blood and lymphatic vessels where the vessels ran parallel to the long axis of the bone; (b) circular vessels where the vessels encircle the bone. These vessels interconnect with (c) short branches with no predominant direction .[58,105,116]

Capillaries are the smallest vessels of the blood circulatory system and form a complex interlinking network. The capillary wall is composed of endothelial cells, a basement membrane, and occasional scattered contractile cells called pericytes. A capillary consist of one, two or three epithelial cells. The capillaries form a dense network of narrow, short tubes measuring from 3-4 µm in diameter (i.e. half the diameter of red blood cells) up to 30-40 µm (these large blood spaces are usually known as sinusoids). On average, capillaries have a diameter of 6-8 µm and are approximately 750 µm-1mm long. Average volume is 40 μm^3 and blood flow 0.1 -0.5m/sec.[43] Oxygen rich blood flows from arterioles into the capillary bed and deoxygenated blood is transported from capillaries to venules. Pressure difference forces the blood from capillary bed to venules. Blood from arterioles travels to terminal arterioles, also called metaarterioles. Metaarterioles have discontinuous layer of smooth muscle cells (in contrast to arterioles). Capillary density in tissue is directly proportional to metabolic activity of the tissue. Capillary density is the highest in the brain, kidneys, liver, heart and muscles and low in bones, fat and fibrous tissue. There are not exact data about capillary density in the periosteum. Periosteal veins have thinner vessel wall with higher quantity of collagen fibers than arteries often leading to luminal collapse during microscopic examination. The layers cannot be strictly differentiated. Periosteal veins contain lesser amount of elastin than periosteal arteries and these fibers are scattered with no predominant direction. Lymphatic vessels have thinner wall than veins and lack distinct layers. The lumen is irregular and its wall consists of endothelial cells surrounded by fibrous tissue. Only larger lymphatic vessels have muscular layer that contains smooth muscle cells in both longitudinal and circular direction.

Periosteocortical (cortical capillary) anastomoses

Periosteal arterioles run longitudinally without a decrease in the diameter and give branches that are directed to bone. Normally, these branches are perpendicular to this main periosteal vessel. In the outer third of the cortex, in the nearest central canal of external osteons they anastomose with medullar system (Fig. 8). The number and the diameter of periosteocortical anastomoses increase progressively from the diaphysis to the metaphysis.[131] In some cases branches of periosteal arteries and arterioles pass through the whole cortex and supply sinusoids and other vessels of medullary system.[75,102] This system represents direct connection of periosteal blood supply with nutritional artery. These periosteocortical arteries have concomitant veins, a system characteristic for all mammals that is responsible for survival of outer cortex when nutritive or medullar blood supply is diminished or blocked.

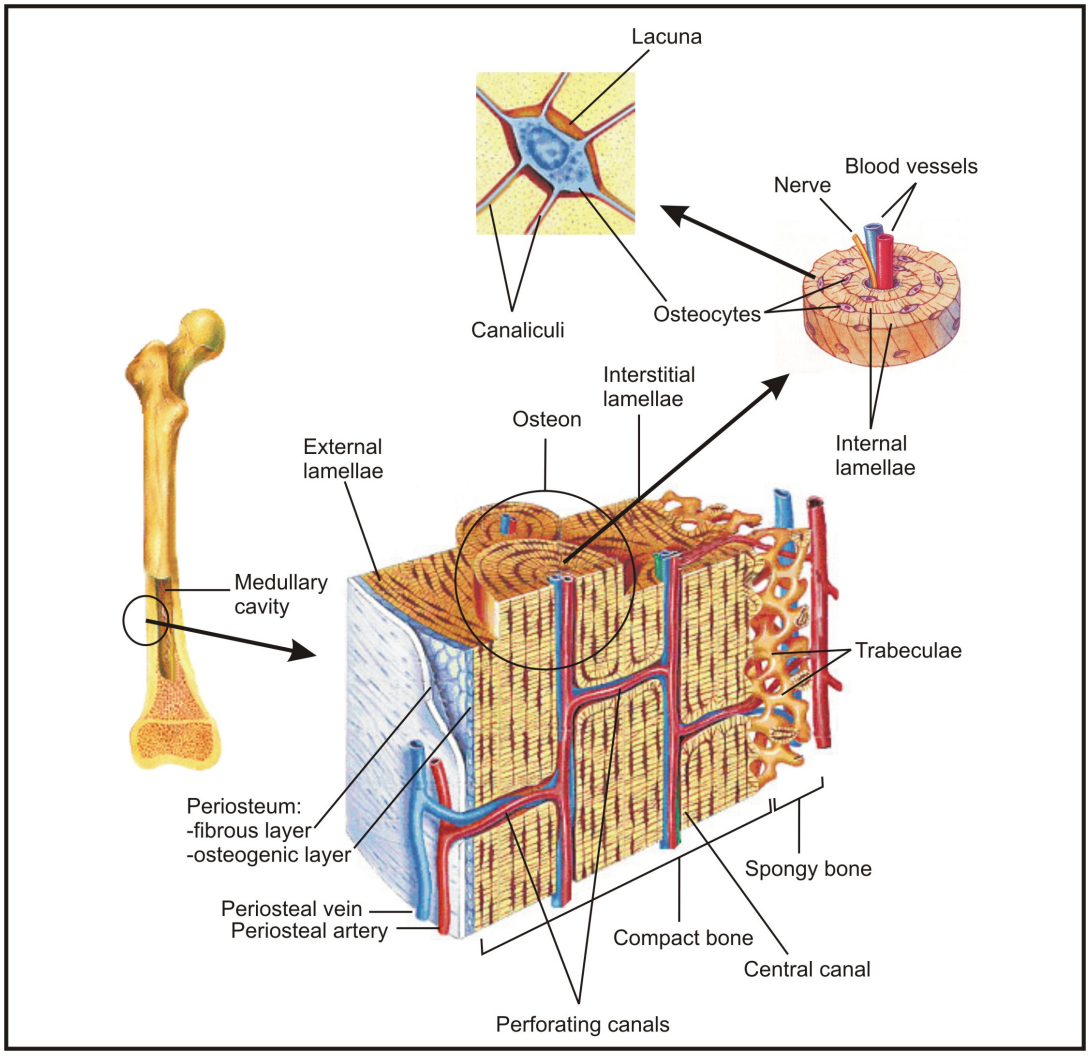

Figure 8 Vascular supply of cortical bone. Periostocortical anastomosis connects periosteal and nutritional artery blood supply.

Musculoperiosteal system

Musculoperiosteal anastomoses with surrounding muscle have a significant role in periosteal callus formation.[84,141] Their role is even more significant in conditions of insufficient intrinsic (nutritive) periosteal circulation. Epimysium is well nourished and fused with fibrous layer of the periosteum in a way that pulling the muscle from the bone resulted in stripping of the periosteum. Blood supply of epimysium is derived from two sources: the main muscular branch (Fig. 9a) and branches of segmental arteries (Fig. 9b). Musculoperiosteal anastomoses can also be divided (according to the size of the vessels) on musculonutritive arteries with concomitant veins and less valuable anastomoses on capillary level.[137]

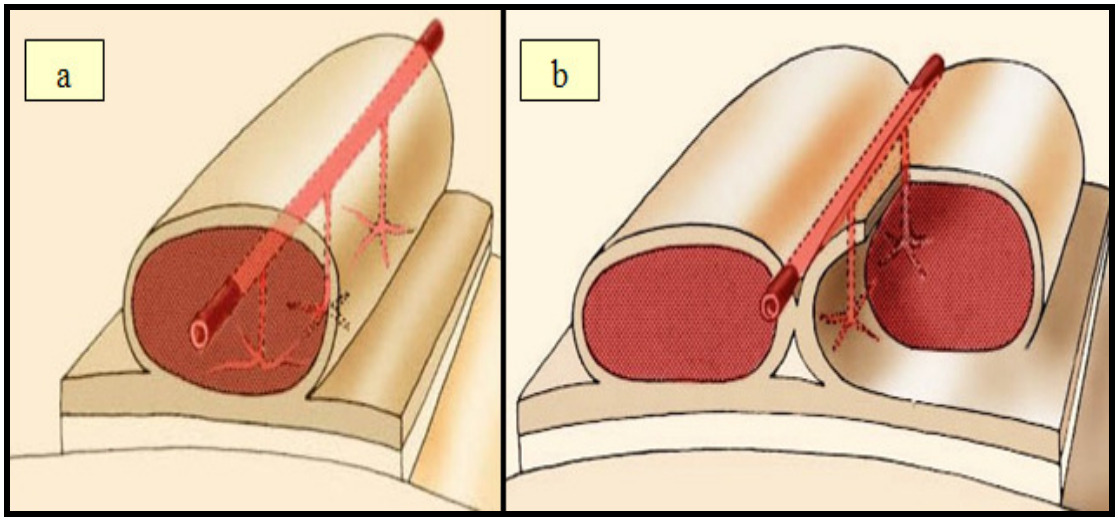

Figure 9 Musculoperiosteal anastomoses, a) main muscular branch, b) branches of segmental artery for epimysium.

Nutritive periosteal system (fascioperiosteal system)

The periosteum is vascularized by several segmental arteries. Distribution of these segmental arteries differs from bone to bone because of different insertion of tendons and fascia.[75] Nutritive arterial system is accompanied by venous system. Every artery is accompanied by two veins.[99] As an example, periosteal circulation of human tibia is presented in detail.[80] Nutritive periosteal circulation of the human tibia is divided into four regions (Fig. 10). These regions are connected at the capillary level. Seventy to eighty percent of cortical blood flow is delivered by periosteal circulation and 90-100 % of venous blood is drained by periosteal circulation depending of the anatomic and bone region.[22,27,116,130,139]

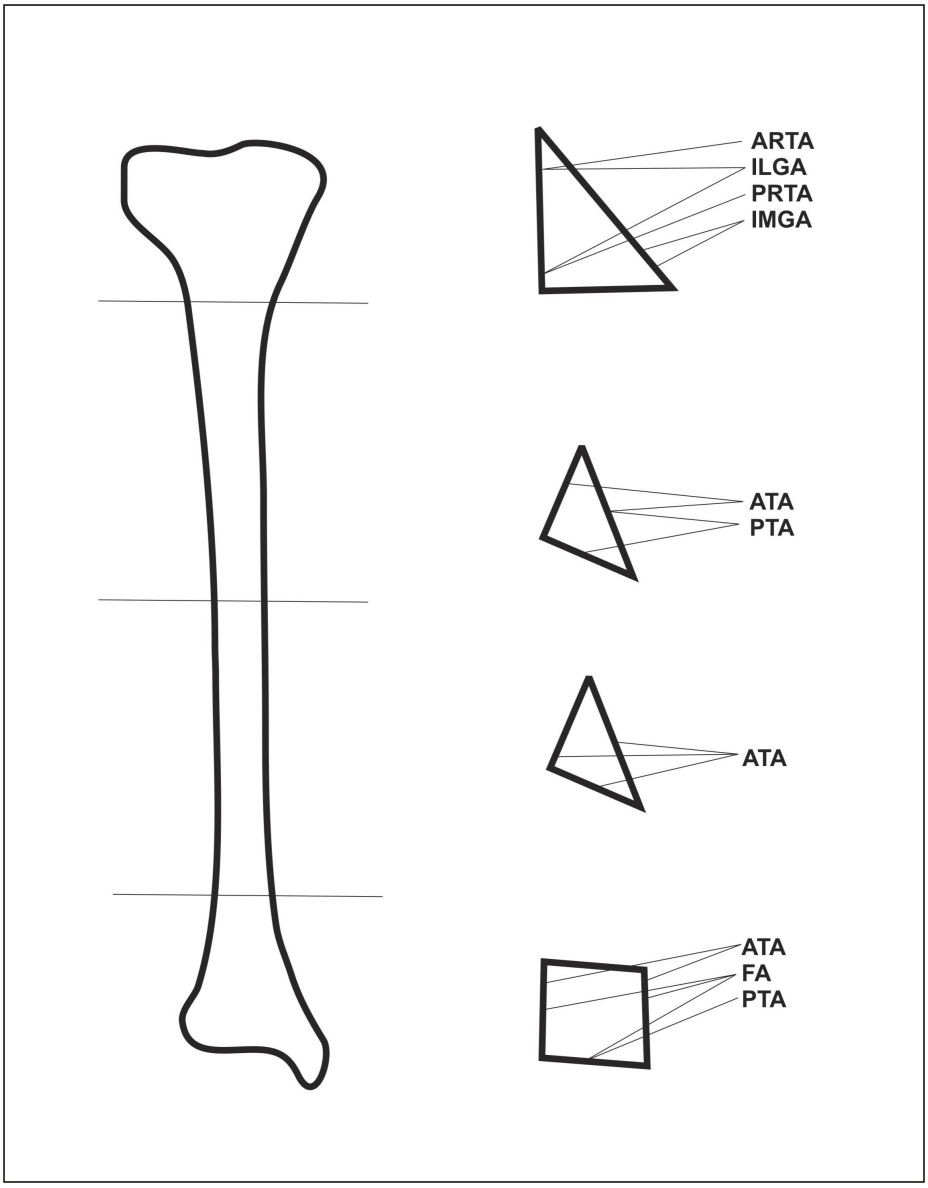

Figure 10 Periosteal arteries of the tibia. Scheme demonstrates sectors being supplied by one or multiple arteries which nourish the periosteum and the outer part of the cortex. ATA=anterior tibial artery; ARTA= anterior recurrent tibial artery; FA=fibular artery; ILGA=inferior lateral genicular artery; IMGA=inferor medial genicular artery, PTA=posterior tibial artery; PRTA=posterior recurrent tibial artery.

The anterolateral sector of the proximal fifth of the tibial periosteum is nourished by recurrent branch of anterior tibial artery (ATA). Anastomoses were found proximally with the lateral inferior genicular artery with branches of the medial inferior genicular artery on the tibial tuberosity and under the distal part of the patellar ligament. In the proximal fifth the latter artery supplies the anteromedial side of the tibia and the medial part of the dorsal side. The lateral part of the dorsal side of the upper fifth of the tibia is nourished by recurrent posterior tibial artery coming from the ATA. At the lateral condyle the supply is supported by the lateral inferior genicular artery from the popliteal artery. Lateral surface of the proximal diaphysis is nourished by periosteal branches from the ATA, mainly running in a transversal or slightly ascending seldom in a descending direction. There are 5-12[80] or 2-8[99] of these

branches. Both authors found that the arterial vessels of the periosteum are accompanied by two veins. Partly the periosteal branches of the proximal diaphysis extend to the medial surface where they merge with periosteal branches of the posterior tibial artery (PTA). In addition, the PTA is giving support to the nutritional artery for supplying the posterior surface. At the level of proximal diaphysis there are vertical and also circular segmental anastomoses of semicircular branches from the ATA and PTA. The nutritional tibial artery often arises from the PTA, seldom from the ATA.

The distal diaphysis is exclusively supplied by branches of the ATA, which form a capillary network with circular and vertical anastomoses. The lateral surface is nourished through periosteal branches which merge on the medial surface with periosteal perforators. The latter originate from the ATA and supply the posterior surface before reaching the medial surface. The total amount of existing periosteal perforators is 2-5.[48,80]

In 2 of 3 cases, the periosteum of the lateral surface around the fibular notch at the caudal fifth of the tibia is nourished by perforators of the fibular artery (FA) which is branching into an ascending and descending branch. The other part of the lateral side is supplied by the periosteal branches from the ATA. In one third, the whole lateral area is nourished by branches of the ATA. In cases when perforators are not developed the ATA gives off a strong branch which copies the course of the first-mentioned artery. The variations of the periosteal perforators are well documented by Hyrtl.[56] The caudal fifth of the posterior surface is mainly supplied by a transversally running periosteal branch of the FA which splits up into multiple small vessels. These capillaries reach the medial surface and anastomose with periosteal branches coming from the lateral surface. Additionally there are branches of the PTA for the supply of the caudal area of the periosteum of the posterior surface. The lateral surface is chiefly nourished by branches of the ATA, whereas the posterior surface is supplied by branches arising from both ATA and PTA and minor parts by the FA and the inferior medial and lateral genicular arteries. Thus, the lateral, as well as the posterior surface, are supplied by direct branches of the major arteries of the lower leg. In contrast the medial surface is nourished only by vessels coming from the lateral and posterior surface, respectively.

From this anatomical consideration it is obvious that anterior tibial artery is of great importance for the arterial supply of the tibial periosteum with an autonomous region at the distal diaphysis. This medial aspect of the third fourth of the tibial periosteum is nourished only by small capillary branches of anterior tibial artery. This is of a significant clinical importance because this area has high incidence of pseudoarthrosis.[16] Periosteal circulation represents a significant part of tibial vascularization and periosteal disruption impairs and diminishes cortical blood supply.[64,138] In short: an osteocorticotomy should neither be made at the distal diaphysis nor in the upper part of the proximal diaphysis, because of disruption of nutritional artery.

Periosteal bone formation during growth

The growth plate components go through a sequential process of cell proliferation, extracellular matrix synthesis, cellular hypertrophy, matrix mineralization, localized vascular invasion and apoptosis. These highly coordinated activities lead to the longitudinal bone

growth and bone formation at the physeal-metaphyseal region by the mechanism of enchondral ossification. The growth cartilage replenishes itself through the germinal zone and is continually replaced by bone at the physeal-metaphyseal junction. The length of the entire bone increases; the physes at either end are displaced progressively further away from the center of the bone, and the physis itself maintains the same height throughout the growth period. At the same time, there is radial growth of the diaphysis and parts of the metaphysis caused by direct apposition of cortical bone by osteoblasts from the inner cambial layer of the periosteum (intramembranous bone formation) (Fig. 3). Apposition of bone around and between periosteal vessels results in formation of periosteal ridges, which, in subsequent phases unite around periosteal vessels thus producing Haversian canal, osteons (Fig. 11).

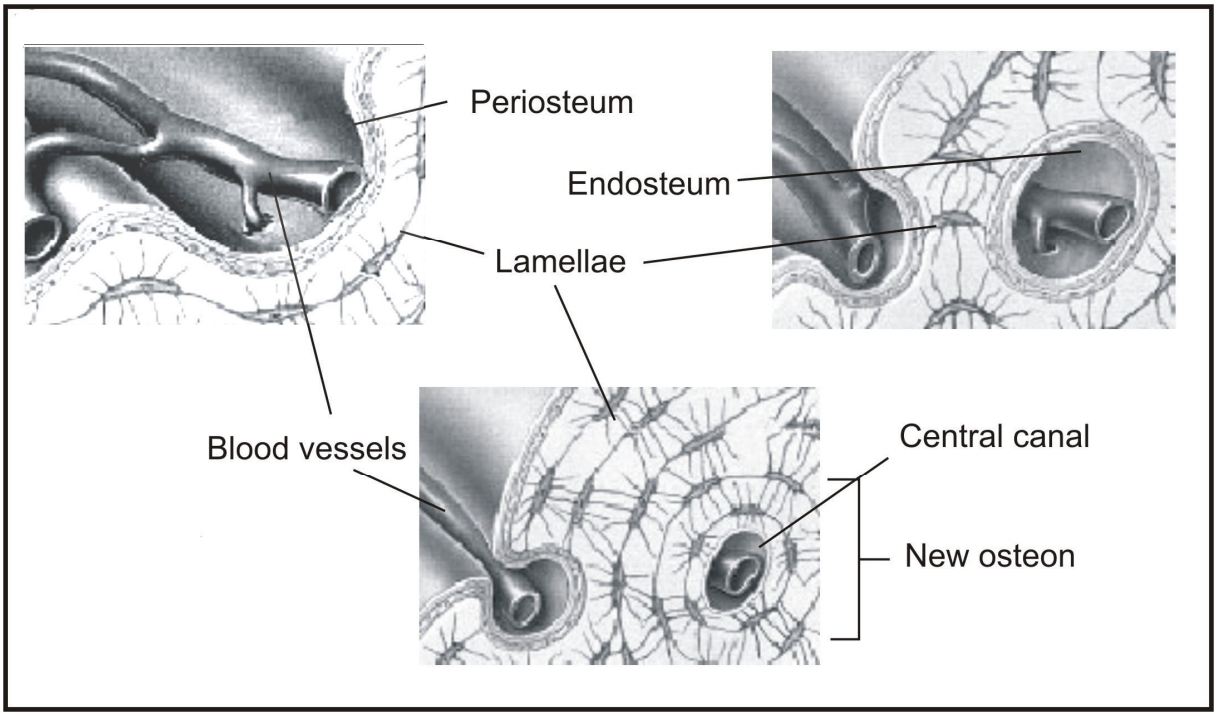

Figure 11 Apposition of bone around periosteal vessels presented in four phases.

There are 16 stages and with several additional substages of long bone and epiphyseal development that represent the timing and coordination of the growth process.[42] Periosteal bone apposition is a cardinal feature of skeletal development. Long bones grow wider as they grow taller, and it is commonly recognized that there is wide individual variation in this process ("big-boned" vs. "small-boned"). In fact, after adjustment for height or weight, there is a wide range in bone size, indicating that periosteal apposition is affected by a distinct set of determinants.[76] In humans, some of the most obvious are gender (males > females) and race (blacks > whites > asians).[73,86,87] Geographical differences in bone size are also marked, even within racial boundaries.[30] Disorders of bone size expansion, such as childhood illness at critical periods of development, have been proposed to contribute to the variation in adult bone strength and fracture likelihood.[17] Animal studies support a positive effect of androgens and a negative effect of estrogens on periosteal bone formation rates.[132] At puberty in males,

the periosteum expands due to androgen action with little change in the endocortical (medullary diameter), so that cortical width increases. At puberty in females, the periosteal expansion ceases. Endocortical (medullary) diameter decreases as the endocortical bone formation occurs. This endocortical contraction contributes 25% of the total cortical thickness.[45] Males and females have the same cortical thickness but the bone diameter is greater in males, conferring greater breaking strength. Thus, reduced cortical thickness may be the result of excessive radial expansion of the endocortical surface relative to the periosteal surface before and during puberty. This may be due to either increased resorption and/or reduced bone formation. A role for insulin-like growth factor 1 (IGF-1) in the regulation of periosteal apposition has long been postulated, especially in concert with sex steroids during puberty.[15] Many other factors are probably involved as well. For instance, mechanical force applied *in vivo* induces the expression of a variety of genes in the periosteum[78] and a rapid transformation of quiescent periosteal surfaces to those on which bone formation occurs.[94] In fact, it has been suggested that the mechanical loading environment is a primary modulator of periosteal apposition.[135] Also, genetic analyses have implicated a variety of chromosomal regions (and genes) in the control of bone size in humans and mice.[62,63] In the light of their effects on bone formation in other skeletal compartments, other lifestyle and environmental factors (e.g., nutrition, alcohol and tobacco use)[108,135] may modulate periosteal bone formation, but their effects have not been well examined.

Periosteal bone formation in adulthood

Animal studies, from rodents to primates, document the persistence of periosteal bone formation throughout life, albeit at a slower rate than during growth, and there is the strong suggestion that bone size may continue to increase during adulthood. At present, most evaluations of change in bone size in humans are small and cross-sectional and are subject to limited power and cohort effects,[69,77,106,107] but some longitudinal studies support the increase in bone size with age.[12,13,69] Mechanical events have usually been assumed to underlie the observation that bone size can increase in adults.[70] One attractive model posits that gradual endosteal bone loss with aging leads to cortical thinning and thus more bending stress on the outer surface of bone, in turn leading to the stimulation of periosteal bone apposition as a biomechanical compensation.[13,69] On the other hand, periosteal expansion also seems to occur in early adulthood, at a time when endosteal resorption has not begun, suggesting that events at the periosteum don't only reflect mechanical influences.[69] Moreover, less loaded bones (metacarpal, skull) also experience periosteal expansion in adults. Although likely important, the relative role of mechanical forces in the determination of periosteal responses are unknown. As during growth, other factors may also influence bone size during aging (nutrition, endocrine factors, lifestyle variation, etc.).

The periosteal effects of selective estrogen receptor modulators or nongenotropic estrogens are unclear. Might other factors known to adversely affect osteoblast viability or bone formation (e.g., glucocorticoids, alcohol, renal dysfunction, vitamin D deficiency, etc.) contribute to a failure of periosteal expansion and increased fracture propensity? Conversely, stimulators of periosteal bone formation should offer new opportunities to improve bone strength. For instance, parathyroid hormone therapy (and even mild hyperparathyroidism) may increase bone size and strength through complex effects on bone forming elements on the periosteal surface.[93] If the postulated sex difference in bone size is a result of androgen action, as some animal studies suggest,[88] it lends support to the potential use of androgenic compounds, acting through an effect on bone size, in the prevention of age-related fracture. The emergence of the

periosteum as a target for pharmacotherapeutics, for instance with parathyroid hormone or androgenic agents, promises to alter approaches to fracture risk reduction.

In most endosteal indices of bone adaptation endosteal adaptation of both the loaded and control tibiae is identical. Moreover, endosteal adaptation did not increase with strain rate. These results of absence of large endosteal adaptive responses, in the presence of large periosteal adaptive responses, are consistent in the literature.[46,71,79,120] That the adaptive response was largely confined to the periosteal surface has significant implications for resistance to bending. For a given amount of bone, bone localized on the surface furthest away from the neutral axis of bending most effectively can resist bending by efficiently elevating the cross-sectional moment of inertia, and that surface represented the periosteal surface.[67]

Periosteal bone resorption

Periosteal resorption is somehow a heretical concept. It is frequently assumed that there is an inexorable expansion of the periosteum through isolated new bone formation, or modeling, and that resorption is rare on the periosteal surface. However, it is unequivocal that periosteal resorption occurs in some situations. Parfitt et al.[93] have pointed out the drift in bone surfaces that accompanies growth, including the dramatic resorption that must occur on the medial ileal surfaces during pelvic enlargement. Analogous events occur in other flat bones (mandible, skull, scapulae). Similarly, longitudinal growth of appendicular bones is accompanied by rapid periosteal resorption of the metaphysis ("waisting") to create the more slender diaphysis. Essentially, the periosteal radius (and size) of the bone shrinks during that process[100] and strength is maintained by simultaneous endocortical bone apposition to form a thickened cortex. While there is simply very little information concerning the presence or absence of resorption on most adult periosteal surfaces, Epker and Frost[39] actually described periosteal resorption (and remodeling) in adults on the surface of ribs almost 40 years ago and Balena et al.[9] examined periosteal remodeling on the surface of the ileum in women. In further studies, the extent of eroded periosteal surface equaled that on the endocortical surface (although there were fewer osteoclasts present on the periosteal surface and in general the remodeling rate was considered much slower than on the endosteal surfaces). It was estimated that the bone formation presented on the periosteum occurred on previously eroded surfaces—in other words, bone formation occurred only as part of remodeling and did not result from modeling. Virtually no other information exists concerning the nature of periosteal remodeling events or their impact on bone health. Nevertheless, there are clear illustrations of this phenomenon. For instance, the alveolar ridge of the mandible can be rapidly lost after tooth loss, which reduces the mechanical forces on it.[7] One example of how the disease can affect the periosteum is that hyperparathyroidism has been classically associated with "subperiosteal" bone resorption. In severe forms, a reduction in mineralized bone size (classically of the phalanges) can be observed radiologically. Whether some or all of this osteoclastic activity originates on the periosteal surface or occurs as a result of exuberant Haversian remodeling (tunneling) within the subperiosteal cortex is unclear. However, the result is a reduction of the effective circumference of bone and arguably its resistance to fracture. To what extent these losses of periosteal bone contribute to the increased fracture risk of advanced hyperparathyroidism is unexplored. In summary, the circumference and to some extent the biomechanical strength of bone should be considered a function of the balance between periosteal bone formation and resorption. However, the rate of periosteal remodeling and the factors that influence it at critical skeletal sites (vertebrae, proximal femur) are unknown.[89]

The Perichondrial Ossification Groove

The perichondrial ossification groove of Ranvier that contains the circumferential bony ring of Lacroix, sometimes referred to as the "bone bark," surrounds the periphery of the growth plate as a differentiated cell and tissue structure with fibers arranged in three directions: vertically, circumferentially, and obliquely. Its components function to contribute to latitudinal growth of the growth plate by appositional addition of chondrocytes, to contain mechanically and support the physes by its outer fibrous sheath, inner osteogenic layer, and bony ring and to elongate cortical intramembranous bone formation by osteoprogenitor cells.[18,19, 50, 57, 66,110,111] The groove of Ranvier surrounds the growth plate and is the specific structural and functional region where the cartilage of the endochondral sequence meets the 2-layered periosteum of the intramembranous sequence. The outer layer of the periosteum is continuous from the diaphysis toward both bone ends enclosing the metaphysis, the growth plate, and inserting into the epiphyseal cartilage beyond the physis. The inner layer of the periosteum with osteogenic cells also covers the growth plate and forms an accumulation of cells at the depth of the groove adjacent to but separate from the cells of the germinal and proliferating layers of the physis. The groove region consists of an outer layer formed by fibroblasts and collagen fibers, which is a continuation of the outer fibrous layer of the periosteum; undifferentiated loosely packed cells, which are cartilage precursors; and a group of densely packed cells that mature into osteoblasts. In those bones or parts of bones in which the diameter of the metaphysis is the same as that of the adjacent diaphysis (i.e., there is no cut-back zone), the inner, cambial layer of the periosteum is continuous into the groove, as is the cortex. Where the cutback zone is prominent, the inner cambial layer and the bone ring of the ossification groove are discontinuous with the inner cambial layer of the periosteum and the diaphyseal cortex. The outer fibrous layer is always continuous and serves as a fibroelastic sheath connecting the epiphyseal cartilage at one end of a bone to the epiphyseal cartilage at the other end and enclosing both physes. The increase in the transverse diameter of the physis is achieved by interstitial growth in the resting cell layer[50,68,104,110] and appositional growth from the region of loosely packed cells (perichondrium) of the groove.[66,110,118,126,127]

Extrinsic Mechanical Effects of the Periosteum on the Growth Plate

The periosteum has an essential role in the formation of cortical bone by its inner osteogenic (cambial) layer. The metaphyseal cortical bone is formed by the coalescence of peripheral endochondral trabecular bone from the physis with intramembranous bone from the inner osteogenic layer of the periosteum.[26]

The outer fibrous layer of the periosteum covers not only diaphyses and metaphyses but also surrounds and mechanically supports the epiphyseal regions of the growing bone, particularly in Ranvier's groove, and eventually attaches into the epiphyseal cartilage beyond the physis. The periosteal sleeve has a strong fibroelastic mechanical effect on the physis. Circumferential cutting of the periosteum reduces the force by 80% needed to produce epiphysiolysis in rats whereas its partial section in the proximal medial tibia causes valgus deformation.[111] Haasbeek et al.[47] showed in two clinical cases that angular deformations occur when the periosteum is thickened adjacent to the physis. Dimitriou et al.[33] compared the effects of surgically induced longitudinal and transverse sectioning of the periosteum, and observed that only the latter increased longitudinal growth of the long bones. These

experimental observations support the mechanical theory that a reduction of tension on the periphyseal region has a beneficial effect on growth whereas increased tension slows growth. After removal of the periosteum of the diaphysis in rats, no notable differences were observed in the heights of the resting, proliferating, and hypertrophic cell layers or in cell proliferation and the rate of longitudinal growth, in comparison with control groups.[49] Growth stimulation in children after femoral shaft fractures is considered to be caused by increased periosteal and periphyseal vascularity affecting the entire bone.[109]

Conflict of interest statement

All the authors declare that there are no financial and personal relationships with other people, or organizations, and that there are no conflicts of interest of any kind.

References

1. Alhlborg H, Johnell O, Turner C et al. Bone loss and bone size after menopause. N Engl J Med 2003;349:327–34.
2. Allen MR, Burr DB. Human femoral neck has less cellular periosteum, and more mineralized periosteum, than femoral diaphyseal bone. Bone 2005;36:311–6.
3. Allen MR, Hock JM, Burr DB. Periosteum: biology, regulation, and response to osteoporosis therapies. Bone 2004;35:1003-12.
4. Anderson CD, Danylchuk K. Bone-remodeling rates of the beagle: a comparison between different sites on the same rib. Am J Vet Res 1978;39:1763–5.
5. Anseroff NJ, Die arterien der langen Knochen des Menschen. Z Anat EntwGesch 1934;103;793-812.
6. Aubin J, Triffit J. Mesenchymal stem cells and osteoblast differentiation. In: Bilezikian J, Raisz LG, Rodan GA. Principles of Bone Biology. San Diego: Academic Press; 2002, 59-81.
7. August M, Kaban LB. The aging maxillofacial skeleton. In: Rosen CJ, Glowacki J, Bilezikian JP. The Aging Skeleton. San Diego: Academic Press; 1999, 359-71.
8. Bagi CM, Wilkie D, Georgelos K et al. Morphological and structural characteristics of the proximal femur in human and rat. Bone 1997;21:261–7.
9. Balena R, Shih M, Parfitt AM. Bone resorption and formation on the periosteal envelope of the ilium: A histomorphometric study in healthy women. J Bone Miner Res 1992;7:1475-82.
10. Banks H. Healing of the femoral neck fracture. Proceedings of the conference on aseptic necrosis of the femoral head, 1964;465–82.
11. Beck TJ, Ruff CB, Bissessur K. Age-related changes in female femoral neck geometry: implications for bone strength. Calcif Tissue Int 1993;53(Suppl. 1):S41–6.
12. Beck TJ, Oreskovic TL, Stone KL et al. Structural adaption to changing skeletal load in the progression toward hip fragility: The study of osteoporotic fractures. J Bone Miner Res 2001;16:1108-19.
13. Beck TJ, Stone KL, Oreskovic TL et al. Effects of current and discontinued estrogen replacement therapy on hip structural geometry: The study of osteoporotic fractures. J Bone Miner Res 2001;16:2103-10.
14. Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 2001;19:180– 92.
15. Bikle D, Majumdar S, Laib A et al. The skeletal structure of insulin-like growth factor I-deficient mice. J Bone Miner Res 2001;16:2320-9.

16. Boer FC, Patka P, Bakker FC, Haarman HJ. Current concepts of fracture healing, delayed unions und nonunions. Osteo Trauma Care 2002;10:1-7.
17. Bradney M, Karlsson MK, Duan Y et al. Heterogeneity in the growth of the axial and appendicular skeleton in boys: implications for the pathogenesis of bone fragility in men. J Bone Miner Res 2000;15:1871–8.
18. Brighton CT: Structure and function of the growth plate. Clin Orthop 1978;136:22–32.
19. Brighton CT, Magnuson PB, Iannotti JP: Growth and development of bone: The growth plate and its abnormalities. In Instructional Course Lectures, Rosemont, American Academy of Orthopedic Surgeons 1992;105.
20. Brighton CT, Lorich DG, Kupcha R et al. The pericyte as a possible osteoblast progenitor cell. Clin Orthop 1992;275:287–99.
21. Bord S, Horner A, Beavan S, Compston J. Estrogen receptors alpha and beta are differentially expressed in developing human bone. J Clin Endocrinol Metab 2001;86:2309–14.
22. Brookes M. The blood supply of bone. London: Butterworth's; 1984, 88.
23. Bruns RR, Palade GE. Studies on blood capillaries I. General organization of blood capillaries in muscle. J Cell Biol 1968;37:244-76.
24. Buckwalter JA, Glimcher MJ, Cooper RR, Recker R. Bone biology I: structure, blood supply, cells, matrix and mineralization. In: Pritchard DJ. Instructional course lectures. Vol 45. Rosemont, IL: American Academy of Orthopaedic Surgeons; 1996:371–86.
25. Burstein, FD, Canalis, RF, Canalis, EM, et al. Scanning electron microscopy and gel electrophoresis of vascularized periosteal autografts. Plast Reconstr Surg 1989;83:500–10.
26. Cadet ER, Gafni RI, McCarthy EF, et al. Mechanisms responsible for longitudinal growth of the cortex: Coalescence of trabecular bone into cortical bone. J Bone Joint Surg 85-A; 2003:1739-48.
27. Chanavaz M. Anatomy and histophysiology of the periosteum: quantification of the periosteal blood supply to the adjacent bone with 85Sr and gamma spectrometry. J Oral Implantol 1995;21:214–9.
28. Chong DA, Evans CA, Heeley JD. Morphology and maturation of the periosteum of the rat mandible. Arch Oral Biol 1982;27:777-85.
29. Cogan DG, Toussaint D, Kuwabara T. Retinal vascular patterns IV. Diabetic Retinopathy. J Diabetic Retinopathy Arch Opthalmol 1961;66:366-78.
30. Crabtree N, Lunt M, Holt G et al. Hip geometry, bone mineral distribution, and bone strength in European men and women: The EPOS Study. Bone 2000; 27:151-9.
31. Crocker DJ, Murad TM, Geer JC. Role of the pericyte in wound healing. An ultrastructural study. Exp Mol Pathol 1970;13:51-65.
32. Diaz-Flores L, Gutierrez R, Lopez-Alonso A et al. Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. Clin Orthop 1992;280–6.
33. Dimitriou CG, Kapetanos GA, Symeonides PP: The effect of partial periosteal division on growth of long bones: An experimental study in rabbits. Clin Orthop 1988;236:265–269.
34. Dixon T, Benjamin J, Lund P et al. Femoral neck buttressing: a radiographic and histologic analysis. Skelet Radiol 2000;29:587–92.
35. Duhamen D, Monceau H. Lettre sur la formation des os dans les animaux, et du bois dans les arbres. Rec période Obs Med Chir Pharm 1757;7:153-60.
36. Dupuytren C. On the injuries and diseases of bones: being selections from the collected edition of the clinical lectures of baron Dupuytren. London, Sydenham Society 1847.
37. Einhorn T. Bone strength: the bottom line. Calcif Tissue Int 1992;51:333–9.
38. Ellender G, Feik SA, Carach BJ. Periosteal structure and development in a rat caudal vertebra. J Anat 1988;158:173– 87.
39. Epker BN, Frost HM. A histological study of remodeling at the periosteal, haversian canal, cortical endosteal, and trabecular endosteal surfaces in human rib. Anat Rec 1965;152:129– 35.
40. Epker BN, Frost HM. Periosteal appositional bone growth from age two to age seventy in man. A tetracycline evaluation. Anat Rec 1966;154:573– 7.
41. Ferretti JL, Frost HM, Gasser JA et al. Perspectives on osteoporosis research: its focus and some insights from a new paradigm. Calcif Tissue Int 1995;57:399–404.
42. Forriol F, Shapiro F. Bone development: interaction of molecular components and biophysical forces. Clin Orthop Relat Res 2005;432:14-33.

43. Freitas RA. Nanomedicine. Vol I: Basic Capabilities. Georgetown: Landes Bioscience 1999;5.
44. Gallie WE, Robertson DE. The periosteum. Can Med Assoc J 1914;41:33-6.
45. Garn SM. The earlier gain and later loss of cortical bone. Springfield: C. C. Thomas, 1970.
46. Gross TS, Srinivasan S, Liu CC, Clemens TL, Bain SD. Noninvasive loading of the murine tibia: an in vivo model for the study of mechanotransduction. J Bone Miner Res 2002;17:493–501.
47. Haasbeek JF, Rang MC, Blackburn N: Periosteal tether causing angular growth deformity: Report of two clinical cases and an experimental model. J Pediatr Orthop 1995;15:677–81.
48. Henle J. Handbuch der Gefäßlehre des Menschen,Vierweg, Braunschweig. 1868.
49. Hernandez JA, Serrano S, Mariñoso ML et al: Bone growth and modeling changes induced by periosteal stripping in the rat. Clin Orthop 1995;320:211–9.
50. Hert J. Growth of the epiphyseal plate in circumference. Acta Anat 1976;82:420–36.
51. Hert J, Hladokova J. Die Gefäßlehre des Menschen. In: Lang J, Wachmuth W. Praktische Anatomie I4, Bein und Statik. Berlin: Springer 1972.
52. Hill EL, Elde R. Distribution of cGRP-, VIP-, SP-, and NPYimmunoreactive nerves in the periosteum of the rat. Cell Tissue Res 1991;264:469–80.
53. Hohmann EL, Elde RP, Rysavy JA et al. Inervation of periosteum and bone by sympathetic vasoctive intestinal peptide-containing nerve fibers. Science 1986;232:868-70.
54. Holtrop ME. The ultrastructure of bone. Ann Clin Lab Sci 1975;5:264-71.
55. Horiuchi K, Amizuka N, Takeshita S et al. Identification and characterization of a novel protein, periostin, with restricted expression o periosteum and periodontal ligament and increased expression by transforming growth factor beta. J Bone Miner Res 1999;14:1239-49.
56. Hyrtl J. Handbuch der topographischen Anatomie und ihrer praktisch medizinisch-chirurgischen Anwendungen, Vol 2. 7th ed. Wien: Braumüller, 1882.
57. Iannotti JP. Growth plate physiology and pathology. Orthop Clin North Am 1990;21:1–17.
58. Iwaku F. Microvasculature of bone and bone marrow. In: Motta PM, Murakami T, Fujita H. Scanning electron microscopy of vascular casts: methods and applications. Amsterdam: Kluwer Academic Publishers 1992;72-86.
59. Jacobsten FS. Periosteum. Its relation to pediatric fractures. J Pediatr Orthop B 1997;6:84-90.
60. Jee WS. Integrated bone tissue physiology: anatomy and physiology. In: Cowin S. Bone mechanics handbook. Boca Raton7 CRC Press; 2001.
61. Jones DB, Nolte H, Scholubbers JG et al. Biochemical signal transduction of mechanical strain in osteoblast-like cells. Biomaterials 1991;12:101–10.
62. R, Turner R, Skinner L et al. Mapping quantitative trait loci that influence femoral cross sectional area in mice. J Bone Miner Res 2002 ;17:1752-60.
63. Koller DL, Liu G, Econs MJ et al. Genome screen for quantitative trait loci underlying normal variation in femoral structure. J Bone Miner Res 2001;16:985-91.
64. Kowalski MJ, Schemitsch EH, Kregor PJ et al. Effect of periosteal stripping on cortical bone perfusion: a laser Doppler study in sheep. Calcif Tissue Int 1996;59:24-6.
65. Lacroix P. Recherches expérimentales sur l'ostéogenèse périostique. Arch Biol 1945;56:185-197.
66. Lacroix P. The Organization of Bone. New York: McGraw-Hill 195l.
67. LaMothe JM, Hamilton NH, Zernicke RF. Strain rate influences periosteal adaptation in mature bone. Med Eng Phys 2005;27:277–84.
68. Langenskiöld A, Heikel HVA, Nevalainen T et al. Regeneration of the growth plate. Acta Anat 1989;134:113–23.
69. Lazenby RA. Continuing pariosteal apposition I: Documentation, hypotheses, and interpretaton. Am J Phys Anthropol 1990;82:461-72.
70. Lazenby RA Continuing periosteal apposition II: The significance of peak bone mass, strain equilibrium, and age-related activity differentials for mechanical compensation in human tubular bones. Am J Phys Anthropol 1990;82:478-84.
71. Lee KC, Maxwell A, Lanyon LE. Validation of a technique for studying functional adaptation of the mouse ulna in response to mechanical loading. Bone 2002;31:407–12.
72. Litvin J, Selim AH, Montgomery M et al. Expression and function of periostin-isoforms in bone. J Cell Biochem 2004;92:1044-106.
73. Looker AC, Beck TJ, Orwoll ES. Does body size account for gender differences in femur bone density and geometry? J Bone Miner Res 2001;16:1291-9.

74. Mach DB, Rogers SD, Sabino MC et al. Originis of skeletal pain; sensory and sympathetic innervation of the mouse femur. Neuroscience 2002;13:155-66.
75. Macnab I, Haas WG. The role of periosteal blood supply in the healing of fractures of tibia. Clin Orth Relat Res 1974;105:27-33.
76. Marshall LM, Lang TF, Cauley JA et al. Femoral bone size among older men and its relation to lean and fat mass. J Bone Miner Res 2002;17(Suppl. 1):S264.
77. Martin RB, Atkinson PJ. Age and sex-related changes in the structure and strength of the human femoral shaft. J Biomech 1977;10:223-31.
78. Matsumoto T, Nakayama K, Kodama Y et al. Effect of mechanical unloading and reloading on periosteal bone formation and gene expression in tail-suspended rapidly growing rats. Bone 1998;22:89S-93S.
79. Meade JB, Cowin SC, Klawitter JJ et al.Bone remodeling due to continuously applied loads. Calcif Tissue Int 1984;36(Suppl. 1):S25–30.
80. Menck J, Bertram C, Lierse W. Sectorial angioarchitecture of the human tibia, Acta Anat (Basel) 1992;143:67-73.
81. Meyrick B, Reid L. Ultrastructural features of the distended pulmonary arteries of the normal rat. Anat Rec 1979;193:71-97.
82. Midura RJ, Su X, Morcuende JA et al.. Parathyroid hormone rapidly stimulates hyaluronan synthesis by periosteal osteoblasts in the tibial diaphysis of the growing rat. J Biol Chem Dec 2003;278:51462–8.
83. Miller SC, Bowman BM, Miller MA et al. Calcium absorption and osseous organ-, tissue-, and envelope-specific changes following ovariectomy in rats. Bone 1991;12:439– 46.
84. Müller ME, Allgöwer M, Schneider R, Willenegger H. Technik der operativen Frakturenbechandlung. Berlin: Springer, 1963;38.
85. Nakahara H, Goldberg VM, Caplan AI. Culture-expanded human periosteal-derived cells exhibit osteochondral potential in vivo. J Orthop Res 1991;9:465–76.
86. Nakamura T, Turner CH, Yoshikawa T et al. Do variations in hip geometry explain differences in hip fracture risk between Japanese and white Americans? J Bone Miner Res 1994;9:1071-6.
87. Nelson DA, Barondess DA, Hendrix SL, Beck TJ. Cross-sectional geometry, bone strength, and bone mass in the proximal femur in black and white postmenpausal women. J Bone Miner Res 2000;15:1992-7.
88. Orwoll ES. Androgens: Basic biology and clinical implication. Calcif Tissue Int 2001;69:185-8.
89. Orwoll ES. Toward an expanded understanding of the role of the periosteum in skeletal health. J Bone Miner Res 2003;18:949-54.
90. Oshima A, Tanabe H, Yan T et al. A novel mechanism for the regulation of osteoblast differentiation: transcription of periostin, a member of the fasciclin I family, is regulated by the bHLH transcription factor, twist. J Cell Biochem 2002;86:792–804.
91. O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS. The chondrogenic potential of periosteum decreases with age. J Orthop Res 2001;19:95–103.
92. Pankovich A. Primary internal fixation of femoral neck fractures. Arch Surg 1975;110:20–6.
93. Parfitt AM. Parathyroid hormone and periosteal bone expansion. J Bone Miner Res 2002;17:1741-3.
94. Pead MJ, Skerry TM, Lanyon LE. Direct transformation from quiescence to bone formation in the adult periosteum following a single brief period of bone loading. J Bone Miner Res 1985;3:647-56.
95. Pfeilschifter J, Diel I, Pilz U et al. Mitogenic responsiveness of human bone cells in vitro to hormones and growth factors decreases with age. J Bone Miner Res 1993;8:707–17.
96. Phemister D. The pathology of ununited fractures of the neck of the femur with special reference to the head. J Bone Joint Surg Am 1939;21:681–93.
97. Pnnarale L, Morini S, Dubaldo E et al. SEM corrosion-cast study of the microcirculation of the flat bone in the rat. Anat Rec 1997;247:426-71.
98. Power J, Loveridge N, Rushton N et al. Evidence for bone formation on the external bperiostealQ surface of the femoral neck: a comparison of intracapsular hip fracture cases and controls. Osteoporos Int 2003;14:141–5.

99. Prescher A. Über eigentümliche Furchenbildungen auf der Facies lateralis tibiae. Verhandlungsband der Anatomischen Gesellschaft, 85. Versammlung Anat Anz Suppl 1991;170:207-8.
100. Rauch F, Neu C, Manz F, Schoenau E. The development of metaphyseal cortex-implications for distal radius fractures during growth. J Bone Miner Res 2001;16:1547-55.
101. Reilly TM, Seldes R, Luchetti W, Brighton CT. Similarities in the phenotypic expression of pericytes and bone cells. Clin Orthop 1998;346:95–103.
102. Reinelander FW. Circulation in bone. In: Biochemistry and physiology of bone, Vol. II. New York: Academic Press 1972;38-46.
103. Rhodin JAG. Histology. A text and atlas. New York: Oxford University Press 1974;196.
104. Rigal WM: The use of tritiated thymidine in studies of chondrogenesis. In: McLean FC, Lacroix P, Buddy AM. Radioisotopes and bone. Oxford: Blackwell Sci Publ 1962;197–225,.
105. Rucker M, Roesken, Vollmar B, Menger DM. A novel approach for comparative study of periosteum, muscle, subcutis and skin microcirculation by intravital fluorescence microscopy. Microvasc Res 1998;56:30-42.
106. Ruff CB, Hayes WC. Subperiosteal expansion and cortical remodeling of the human femur and tibia with aging. Science 1982;217:945–8.
107. Seeman E. From density to structure: Growing up and growing old on the surfaces of bone. J Bone Miner Res 1997;12:509-21.
108. Seeman E. An exercise in geometry. J Bone Miner Res 2002;17:373-80.
109. Shapiro F. Fractures of the femoral shaft in children. The overgrowth phenomenon. Acta Orthop Scand 1981;52:649–55,.
110. Shapiro F, Holtrop ME, Glimcher MJ. Organization and cellular biology of the perichondrial ossification groove of Ranvier. J Bone Joint Surg 1977;59A:703–23.
111. Shapiro F. Pediatric orthopedic deformities. Basic science, diagnosis, and treatment. San Diego: Elsevier 2002.
112. Shea JE, Vajda EG, Bloebaum RD. Evidence of a hypermineralised calcified fibrocartilage on the human femoral neck and lesser trochanter. J Anat 2001;198:153– 62.
113. Sheng M, Baylink D, Beamer W et al. Histomorphometric studies show that bone formation and bone mineral apposition rates are greater in C3H/HeJ (high-density) than C57BL/6J (low-density) mice during growth. Bone 1999;25:421–9.
114. Simon TM, Sickle DC, Dennis H. Cambium cell stimulation from surgical release of the periosteum. J Ortho Res 2003;21:470-80.
115. Sims DE. The pericyte – A review. Tissue Cell 1986;18:153-74.
116. Simpson AH. The blood supply of periosteum. J Anat 1985;140:697-70.
117. Smith Jr RW, Walker RR. Femoral expansion in aging women: implications for osteoporosis and fractures. Science 1964;145:156–7.
118. Solomon L. Diametric growth of the epiphyseal plate. J Bone Joint Surg 1966;48B:170-7.
119. Squier CA, Ghoneim S, Kremenak CR. Ultrastructure of the periosteum from membrane bone. J Anat 1990;171:233–9.
120. Srinivasan S, Weimer DA, Agans SC et al. Lowmagnitude mechanical loading becomes osteogenic when rest is inserted between each load cycle. J Bone Miner Res 2002;17:1613–20.
121. Stephen E. Asmus, Sarah Parsons, and Story C. Landis developmental changes in the transmitter properties of sympathetic neurons that innervate the periosteum J Neurosci 2000;20:1495–504.
122. Szechinski JW, Grigorian MA, Grainger AJ et al. Femoral neck and intertrochanteric fractures: radiographic indicators of fracture healing. Orthopedics 2002;25:1365–8.
123. Takeshita S, Kikuno R, Tezuka K, Amann E. Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. Biochem J 1993;294:271–8.
124. Tang XM, Chai BF. Ultrastructural investigation of osteogenic cells. Chin Med J (Engl) 1986;99:950-6.
125. Taylor JF. The periosteum and bone growth. In: Hall BK. Bone growth VI. Boca Raton7 CRC Press 1992.
126. Tonna EA. The cellular complement of the skeletal system studied autoradiographically with tritiated thymidine (H3TDR) during growth and ageing. J Biophys Biochem Cytol 1961;9:813–24.

127. Tonna EA, Cronkite EP. The periosteum. Autoradiographic studies on cellular proliferation and transformation utilizing tritiated thymidine. Clin Orthop 1963;30:218– 33.
128. Tonna EA. Response of the cellular phase of skeleton to trauma. Periodontics 1965;4:105-14.
129. Tonna EA. Electron microscopy of aging skeletal cells. III Periosteum. Laboratory Investigation.1975;61:609-32.
130. Triffitt PD, Cieslak CA, Gegg PJ. A quantitative study of the routes of blood flow to the tibial diaphys after an osteotomy. J Orthop Res 1993;11:49-57.
131. Trueta J. Blood supply and the rate of healing of tibial fractures. Clin Orthop Relat Res 1974;105:11-27.
132. Turner RT, Wakley GK, Hannon KS. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. J Orthop Res 1990;8:612-7.
133. Urist MR, DeLange RJ, Finerman GAM. Bone cell differentiation and growth factors. Science 1983;220:680-6.
134. Vajda E, Bloebaum R. Age-related hypermineralization in the female proximal human femur. Anat Rec 1999;255:202–11.
135. van der Meulen MCH, Ashford MW, Kiratli BJet al. Determinants of femoral geometry and structure during adolescent growth. J Orthop Res 1996;14:22-9.
136. Wheater PR, Gurkitt HG, Daniesls VG. Functional histology. New York: Churchill Livingstone 1987;149.
137. Whiteside LA, Lesker P. The effects of extraperiosteal and subperiosteal dissection. I. On blood flow in muscle. J Bone Joint Surg 1978;60:23-6.
138. Whiteside LA, Lesker P. The effects of extraperiosteal and subperiosteal dissection. II. On fracture healing. J Bone Joint Surg 1978;60:26-9.
139. Wilson JW, Rhinelander FW. Bone supply to developing, mature and healing bone. In: Sumner-Smith. Bone in clinical orthopedics: A study of clinical osteology. Philadelphia: WB Saunders 1986:57-63.
140. Zagba-Mongalima G, Goret-Nicaise M, Dhem A. Age changes in human bone: a microradiographic and histological study of subperiosteal and periosteal calcifications. Gerontology 1988;34:264– 76.
141. Zucman J. Studies on the vascular connections between periosteum, bone and muscle. Br J Surg 1960;48:324-8.