Periosteum: Biology, regulation, and response to osteoporosis therapies

Matthew R. Allen [1], Janet M. Hock [1,2] and David B. Burr [1,3]

[1]Indiana University School of Medicine, Anatomy & Cell Biology, Indianapolis, IN, USA.

[2]Indiana University School of Medicine, Department of Periodontics, Indianapolis, IN, USA.

[3]Indiana University School of Medicine, Department of Orthopaedic Surgery, Indianapolis, IN, USA

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Corresponding author

Matt Allen, PhD

Department of Anatomy and Cell Biology

Indiana University School of Medicine

Medical Science Building 5045

Indianapolis, IN 46202

Email: matallen@iupui.edu

Phone: (317) 274-2308

Fax: (317) 278-2040

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Abstract

Periosteum contains osteogenic cells that regulate the outer shape of bone, and work in co-ordination with inner cortical endosteum to regulate cortical thickness and the size and position of a bone in space. Induction of periosteal expansion, especially at sites such as the lumbar spine and femoral neck, reduces fracture risk by modifying bone dimensions to increase bone strength. The cell and molecular mechanisms that selectively and specifically activate periosteal expansion, as well as the mechanisms by which osteoporosis drugs regulate periosteum remain poorly understood. We speculate that an alternate strategy to protect human bones from fracture may be through targeting of the periosteum, either using current or novel agents. In this review, we highlight current concepts of periosteal cell biology, including their apparent differences from endosteal osteogenic cells, discuss the limited data regarding how the periosteal surface is regulated by currently approved osteoporosis drugs, and suggest one potential means through which targeting periosteum may be achieved. Improving our understanding of mechanisms controlling periosteal expansion will likely provide insights necessary to enhance current and develop novel interventions to further reduce the risk of osteoporotic fractures.

Introduction

Osteoporosis drugs reduce fracture risk at clinically relevant sites, such as the femoral neck and lumbar spine in postmenopausal women. Although increased bone mineral density (BMD) contributes significantly to the reduced fracture risk [1], statistical analyses show the protective effects of antiresorptive and anabolic drugs cannot be explained by increased BMD alone [2, 3]. The effects of osteoporotic drugs on cortical bone surfaces, which dictate bone geometry and thereby significantly influence overall strength, are less well understood. Because femoral neck fractures initiate in cortical bone [4], and greater cortical bone mass may explain the higher resistance to vertebral fracture in males [5, 6], cortical bone biology clearly plays a major role in fracture prevention. Periosteal expansion of the cortical shell significantly increases bone strength, independent of increases in areal bone mineral density [7, 8]. This holds true even for bones composed predominantly of trabecular bone, such as the femoral neck and lumbar vertebrae [9]. Gaining a better understanding of how current osteoporosis drugs regulate cortical bone biology, especially the preservation and expansion of periosteal surfaces, is critical to discovery of new therapeutic regimens to reduce fractures.

Periosteum is a thin layer of osteogenic and fibroblastic cells in a well-developed nerve and microvascular network, located along the periosteal cortex of cortical bone (Fig. 1). Because there are ligament and tendon muscle attachments, and fibrocartilage, on some areas of the periosteal surface, the different physical environments to which periosteal cells are exposed is quite unlike that of the more frequently studied endosteal cells which are bathed in hematopoietic marrow. Compared to endosteal osteoblasts, periosteal osteoblasts exhibit greater mechanosensitivity to strain [10], a lower threshold of responsiveness to osteogenic compounds such as parathyroid hormone [11], higher levels of expression of

proteins such as periostin [12-14], and more estrogen alpha receptors [15]. These differences in threshold sensitivity to physical, hormonal and mechanical stimuli may underlie the differences in periosteal and endosteal surface responses to therapy [16]. More extensive data are needed to fully characterize and understand the reasons for any difference at the cellular level. Once this is accomplished, then periosteal cells can be targeted therapeutically.

Our knowledge of the effects of approved osteoporosis drugs on cortical bone biology is limited. Anti-resorptive and anabolic osteoporotic drugs may regulate periosteal cells differently than endosteal cells. For mechanical reasons, periosteal stimulation may provide better anti-fracture efficacy than agents that primarily target endosteal and trabecular cell populations [17, 18]. We speculate that an alternate strategy to protect human bones from fracture may be through targeting of the periosteum, either using current or novel agents. In this review, we highlight current concepts of periosteal cell biology, including their apparent differences from endosteal osteogenic cells, discuss the limited data regarding how the periosteal surface is regulated by currently approved osteoporosis drugs, and suggest one potential means through which targeting periosteum may be achieved.

Periosteum anatomy

Periosteum covers the external surfaces of most bones, to serve as a transitional region between cortical bone and the overlying soft tissue or musculature. Long bones exhibit a continuous periosteal surface, yet this surface may not be covered by an intact periosteum. Periosteum is absent from articular surfaces, tendon insertions, or sesamoid bone surfaces [19], and is present in locations at high risk for fracture, such as femoral neck, distal radius, and vertebrae. The existence of periosteum at the femoral neck has been questioned; early observational [20, 21] and histological [22] studies suggested the femoral neck lacked a

periosteal cambium layer. More comprehensive and recent histological studies show that periosteum is present on human femoral neck surfaces [23-26], and in some cases, a thin cambium layer with osteoblasts can be observed in discrete locations (Fig. 2) [26, 27].

Periosteum is composed of two distinct layers when viewed histologically (Fig. 1), and of up to five distinctly different functional regions when dissociated enzymatically and cultured [28-30]. Anatomically, the outer more "fibrous" layer of periosteum is composed of fibroblasts, collagen, and elastin fibers [31] along with a distinctive nerve and microvascular network [32, 33]. The inner "cambium" layer, positioned in direct contact with the bone surface, is highly cellular. It contains adult mesenchymal progenitor cells, differentiated osteogenic progenitor cells and osteoblasts [34], fibroblasts [35], as well as microvessels [32] and sympathetic nerves [33]. Sympathetic nerve density is significantly higher compared to the endosteum [36], but the relevance of this difference in terms of a contribution to regulation of periosteum homeostasis and bone formation is not known.

Osteoblasts of the cambium layer are cuboidal in immature bone, becoming more elongated [32] and fewer in number [37] with maturity. This reduction in osteoblast number may contribute to the apparent atrophy and thinning of the cambium layer that occurs with age [38]. Fibroblasts within the cambium layer are smaller and more isodiametric than those in the outer fibrous layer, which have more typical (elongated) fibroblast characteristics [35]. Periosteal fibroblast number and fibrous layer thickness decrease with age [37], although atrophy of the fibrous layer is less than that of the cambium layer [32, 38]. Vessel density throughout the periosteum also declines with age [32], but retains the capacity to increase when activated by mechanical loading or fracture repair [32]. These age-induced changes may help explain why periosteal cells from older subjects fail to form mineralized nodules in culture [39], and why periosteal bone formation rate [40], and

responsiveness to hormones and cytokines [41] declines with age. Whether such changes in periosteal cells are also due to age-related changes in circulating hormones known to influence the periosteum, such as growth hormone and sex steroids [42], deserves further study.

Due to its high vascularity, the periosteum contains an abundance of endothelial pericytes [43]. Pericytes are cells in physical contact with capillary endothelial cells, with the ability to differentiate into numerous cell types, including osteoblasts, under appropriate culture conditions [29, 44]. These cells may serve as a supplementary source of osteoprogenitor cells [43] and may be more important in periosteal bone formation, due to their greater abundance in periosteum [45], than in endosteal bone surface apposition [29]. Cultured pericytes mineralize in vitro [45] and synthesize the osteoblast marker, alkaline phosphatase [45], as well as bone matrix proteins, including osteocalcin [32, 45], osteonectin [32], osteopontin [32], and bone sialoprotein [32]. These cells form an osteogenic tissue that mimics bone-derived tissue, both spatially and temporally [32] and responds to osteogenic stimuli, such as BMP and parathyroid hormone [44]. A potential role for pericytes as a source of osteoblasts in periosteum has not been investigated.

Site-specific differences in periosteum anatomy/activity clearly exist throughout the skeleton. It is well know that the calvarial periosteum is uniquely regulated compared to the axial skeleton (see below), and that cellular periosteum is sparse at the femoral neck [24]. As the femoral neck increases periosteal dimensions with age [46, 47], the consequences of having sparse periosteum are not clear. There are few studies that specifically address the site-specific differences [48-50] yet clear differences (> 3 fold) in periosteal bone formation rates exist among skeletal sites (Fig 3). Such varying rates suggest periosteum anatomy/regulation may differ throughout the axial skeleton. Further

investigation of such site-specific differences is essential as it is possible that targeting the periosteum may benefit only certain locations.

Periosteal cells are unique from other osteogenic cell populations

Most data detailing periosteal cell responses have been derived from cultures of calvarial-derived cells, such as the MC3T3.E1 cell lines, primary calvarial cells or calvaria organ cultures. Abuin and Triffitt [34] present an excellent review of the genetic regulation and hormonal responsiveness of these cells. Although the embryonic mouse calvarial cell line (MC3T3.E1) has been studied in great detail, its validity as a model for periosteal osteoblast responses of appendicular and axial bones has not been adequately investigated. Calvarial periosteum appears to be regulated differently from the periosteum of appendicular and axial bones [51], and it is important to clarify whether MC3T3.E1 periosteal cells predict generic periosteal responsiveness in culture, or are more representative of specific calvarial periosteal responses. Furthermore, if this cell line is to be used as a prototype periosteal cell model, it will be important for scientists in the field to reach consensus on the characteristics of a reproducible phenotype, and standardized culture conditions, as these parameters currently differ for the MC3T3.E1 cell line among laboratories [52].

Of studies using periosteal cells from appendicular or axial bones, few have directly compared the response of periosteal and endosteal cells. Differences between periosteal and endosteal cells are qualitative and quantitative, and range from patterns of growth in culture [53] to the response to mechanical [10] and pharmacological stimuli [11]. Periosteal cells divide more rapidly and mineralize in a more random pattern in vitro [53]. When exposed to physiological levels of mechanical strain (3000με), periosteal cells increased proliferation and PGE_2 production while osteoblasts of endosteal origin failed to respond [10]. Cultured periosteal cells respond to PTH at a lower threshold, exhibiting a 7-fold increase in bone

matrix protein production compared to non-treated control cultures; cultured endosteal cells inhibit bone matrix protein production when exposed to PTH [11]. Collectively, the data support the idea that osteogenic cells display site-specific characteristics although the limited studies make definitive conclusions difficult. If such differences are confirmed, it will be essential to define how they translates to in vivo responses.

Effect of approved osteoporosis drugs on periosteal expansion

Current pharmacological interventions include anabolic and anti-resorptive agents. Both modes of treatment reduce risk of osteoporotic bone fracture, in part by increasing bone density. Anabolic agents, such as PTH, increase bone modeling [54] and remodeling [55]. Anti-resorptive agents, such as the bisphosphonates (e.g. alendronate, risedronate, ibandronate, incadronate, or pamidronate), and estrogenic compounds (estrogen, raloxifene) suppress bone remodeling through suppression of osteoclast resorption and increased osteoclast apoptosis. The extent to which these various agents have surface-specific effects and share common mechanistic pathways on the periosteal surface has not been studied in depth.

Dual energy x-ray absorptiometry (DXA) is the most common form of skeletal assessment in humans with differences in total cross-sectional area assumed to be related to periosteal apposition. The limited resolution of DXA is well known [56], and may account for the high variability among studies investigating pharmacological interventions in humans. Despite limitations, the paucity of data on pharmacological effects on periosteal bone in humans necessitates generalization to be drawn using such data.

Once daily parathyroid hormone (PTH) treatment increases cortical bone width through preferential modeling on both periosteal and endosteal surfaces [56-60]. Cross sectional clinical studies using DXA document significant increases in vertebrae [61] and

radius [62] cross sectional area following 12-18 months of PTH treatment in post-menopausal women. Histological [54, 63-65] and microCT [65] data document PTH-induced increases of cortical wall thickness. Femoral neck cortical bone volume increased over 18 months with PTH treatment in post-menopausal women [66]. Data from paired iliac crest biopsies suggests this to be the result of both endocortical and periosteal surface formation [54]. Recent clinical trials document an attenuated BMD increases if anti-resorptive agents are given prior to or simultaneously with PTH (1-34), suggesting remodeling accounts for a significant portion of PTH-induced benefits [66-68]. Blunted effects of PTH were noted in cancellous bone of animal previously treated with anti-resorptive agents [69] whereas other animal studies show resorption is not necessary for increased bone formation on cancellous bone [70, 71]; no such data exist on cortical bone. More detailed studies that clearly show the surface- and time-specific effect of PTH have been carried out in mice [60, 72]. Paradoxically, continuous exposure to PTH and hyperparathyroidism in humans [59] and normal and genetically modified mice stimulates periosteal surfaces, but fails to stimulate endocortical surfaces [73-79]. Even more puzzling, constitutive activation of the PTH1 receptor in genetically modified mice inhibits periosteal and endocortical bone formation, but stimulates trabecular bone formation [80]. Continuation of this work using genetically modified mouse models to elucidate the surface-specific role of PTH is essential, as is more focused research on the periosteal surface response to PTH in humans.

The anabolic effect of recombinant human growth hormone (rhGH) on periosteal surfaces is well established in animals models [81], [82, 83] but remains unclear in humans due to limited studies. Clinical trials using rhGH have documented increased cross sectional area of the rib [84], lumbar vertebrae and femoral neck [85] using longitudinal biopsy and

DXA analyses. The interdependence of growth hormone and insulin-like growth factor I (IGF-I) versus GH-independent effects of IGF-I make it difficult to independently assess the contribution of each compound to periosteal apposition. IGF-I and it's interaction with the six known binding proteins influence periosteal geometry based on animal models [86, 87], [88]. No studies to date have assessed IGF-I's influence on periosteal apposition in humans although low-dose recombinant human IGF-I treatment for one month significantly enhanced bone formation biomarkers in women [89]. When given at bone effective doses, the side-effects of GH, and to a lesser degree IGF-I, have limited the advancement of human studies to assess their use as osteoporosis therapies. Recently some investigators have suggested such treatments could proceed with thorough oversight [90]. More research is needed to understand how rhGH and its intermediaries regulate periosteal biology in humans to determine if these are prototype agents to selectively stimulate periosteal expansion and protect against osteoporotic fracture.

Few data document if bisphosphonate-induced reductions in bone remodeling impact periosteal expansion. One year of high dose bisphosphonate treatment results in significantly higher rib cross-sectional area (compared to controls) in dogs although periosteal bone formation rate was not different at sacrifice [91]. This suggests the increased rate of periosteal apposition was transient and occurred early during treatment. Neither tibial diaphyseal periosteal perimeter of primates [92] nor iliac crest cortical width of women were significantly altered [93] following prolonged bisphosphonate treatment. Evidence suggests positive effects of bisphosphonates on osteoblasts in vitro [94-96], so the selective effect of these drugs on periosteal modeling/remodeling should be assessed. The periosteal surface displays evidence of bone resorption and therefore undergoes remodeling [8, 97]. If the majority of apposition on the periosteal surface is remodeling driven, the

potential benefits of bisphosphonates on osteoblasts would not likely translate into new bone formation although they could prevent some loss from this surface. If periosteal apposition occurs via modeling processes, and if bisphosphonates suppress osteoblast apoptosis [98, 99], the benefits to bone strength by periosteal mechanisms could be significant.

Estrogen inhibits periosteal expansion while estrogen deficiency stimulates periosteal expansion in animals [100-102]. Androgens stimulate periosteal apposition [103] while androgen deficiency reduces periosteal apposition rates [101, 104, 105]. Periosteal cells express both estrogen (alpha and beta) [15] and androgen receptors [106] as well as numerous enzymes important for inter-conversion of sex steroids (i.e. aromatase, 5-α reductase) [107, 108]. The estrogen receptor-alpha (ERα), is more highly expressed in cortical bone [15], and appears a major regulator of periosteal apposition in males and females. Mice lacking ERα receptors exhibit reduced periosteal diameter [109, 110] and an attenuation of loading-induced periosteal apposition [111]. Osteoblasts lacking ERα do not respond to strain in vitro [112]. Animals and cells lacking ERß are minimally affected with respect to periosteal geometry and cellular activity. The absence of androgen receptors (AR) abrogates testosterone-induced increases in periosteal bone formation [113] while mice overexpressing AR exhibit increased periosteal formation rate [114]. These animal and cell culture studies clearly document the influence of sex steroids on periosteal cell activity, the effects of these hormones on human periosteal bone are less clear.

Pubertal changes in sex steroids account for the sexual dimorphism in human periosteal geometry [115, 116]. How age-induced changes in exogenous sex steroid levels influence periosteal expansion during the adult years, along with the effect of pharmacological supplementation/replacement, are unclear. Reduced serum estrogen levels occurring during menopause are associated with periosteal expansion and concomitant loss

of bone from endocortical and trabecular surfaces [7]. Estrogen replacement or hormone replacement therapy increases periosteal apposition. Postmenopausal women taking estrogen therapy for one year increased vertebral cross sectional area [61], and show a trend toward increased iliac crest cortical width [117]. Cross sectional area of the femoral neck and midshaft increased more in estrogen-treated postmenopausal women than in controls [118]. There are no known data on periosteal changes with androgen treatment in humans. Limited data suggest selective estrogen receptor modulators (SERMs) have little or no effect on periosteal apposition in humans. Iliac crest biopsy data document a non-significant increase in cortical bone width after two years of raloxifene treatment, compared to a decrease in placebo treated subjects [119]. Clearly sex steroids and their interaction with osteoblast receptors influence periosteal apposition. Elucidating the mechanisms of though which these interactions regulate periosteal biology may lead to novel drug targets for stimulating periosteal expansion.

Two important concepts influence the value of pharmacological stimulation of periosteal apposition. First, we need to understand the comparative extent to which periosteal apposition relies on modeling versus remodeling. Illiac crest data document both modeling and remodeling on the periosteal surface in healthy women [97]. Differences in the relative contribution of remodeling on cortical periosteal surfaces may determine the relative benefit of anabolic and anti-resorptive treatments. Second, it is important to understand the conditions under which periosteal apposition is related to endosteal resorption. It is hypothesized that the loss of endocortical surface bone leads to higher stress on the remaining bone, especially on the periosteal surface where stresses are highest in bending, resulting in periosteal formation to normalize the stress [120]. Anti-resorptive agents that reduce endocortical bone loss, and anabolic agents that increase endocortical

formation, could reduce the need for periosteal apposition if mechanical compensation is absolute. It is likely that the magnitude of mechanical compensation depends on initial bone size. Smaller bones exhibit more periosteal apposition in response to an equal absolute amount of endocortical bone loss in larger bones [121]. Interactions between periosteal and endosteal cortical bone surfaces, and the role that mechanical compensation plays in periosteal expansion, necessitates more in-depth study.

Possible mechanisms to target periosteal bone formation.

In vivo studies of animals and post-menopausal women have revealed differences in the osteogenic response on periosteal and endosteal surfaces, indicating a potential to preferentially target the periosteal surface cells and increase bone circumference, thereby reducing the risk of osteoporotic fracture. Selective targeting of the periosteum requires we identify genes and proteins unique to periosteum, or present in greater concentrations in periosteum. Recently, seven chromosomes that contain quantitative trait loci for periosteal circumference in genetically altered mice were identified [122]. These data provide a starting point from which to increase our understanding of the genetic control of periosteal dimensions.

The relatively small quantity of periosteum at a given site other than calvaria, and the difficulties in isolating relevant periosteal cells for such studies in animals and humans, present hurdles that may be overcome by the use of technologies such as laser dissection microscopy combined with molecular biology assays and tissue arrays, such as those used in cancer. It is important to determine the relative extent to which animal models to predict human periosteal cell responses. New data concerning periosteal adaptations in humans is essential to improve our understanding of periosteal biology. More detailed cortical bone analysis of iliac crest biopsies is necessary, beyond simply measuring cortical thickness. If

we are to gain a better understanding of how pharmacological interventions influence the periosteal bone surface, more in-depth analyses should be undertaken including dynamic bone formation assessment. The ability of the periosteum of the iliac crest to predict periosteal responses at sites of greater osteoporotic fracture risk needs to be clarified.

To date, only one protein, periostin, is present in greater abundance in periosteum. Periostin is localized predominantly in preosteoblasts, and secreted into the extracellular matrix [12]. Periostin, originally termed OSF-2 [14], is highly expressed in the periosteum cambium layer and in the mouse periosteal calvarial cell line, MC3T3.E1 during proliferation [12]. Expression of periostin is increased 4-fold within three days of fracture [123]. The transiently higher expression of periostin during osteoprogenitor proliferation and abnormal osteoblast proliferation, and the decline in expression as differentiation progresses, need to be better understood within the context of periosteal biology. Expression of periostin is negatively regulated by 1,25-$(OH)_2$-D_3 [14] and positively regulated by TGF-ß [12, 14]. Through interaction with the promoter of a transcription factor Twist, which is important for osteogenesis, periostin acts as a negative regulator for osteoblast differentiation [13]. Further work is necessary to determine if the periostin-null mouse can be used as a model to study periosteal adaptations.

Conclusions

This review takes a somewhat different approach than other recent reviews of periosteal biology [8, 124-126] by focusing on the implications of the anatomical structure of the periosteum and pointing out the limited data available from clinical trials with respect to the effects of currently approved osteoporosis pharmaceuticals. Specifically, periosteal cells appear to differ from endosteal cells; each cell population responds differently both qualitatively and quantitatively to a wide variety of hormones and growth factors. We

suggest, after considering the limited published data of therapeutic interventions for osteoporosis, that substantial work should be undertaken to assess how current drugs influence periosteal cells. We speculate there are selective and specific drug targets within the periosteum that can be activated independently of endocortical or trabecular surfaces. Expanding the periosteal perimeter would represent a novel mechanism to dramatically improve bone strength and reduce fracture risk, independent of the well-accepted effects of increasing bone density.

Figure captions

Figure 1. Periosteal covering of the human femoral midshaft. Note the abundance of cells (arrowheads) near the periosteal surface comprising the cambium layer. Section is from an 81 year old female cadaver stained with Massons trichrome. Original magnification x 400, bar = 25μm.

Figure 2. Periosteal covering of the human femoral neck. Note the sparseness of cells (arrowheads) near the periosteal surface as well as the abundant mineralized tissue (M) near the periosteal surface. Section is from an 81 year old female cadaver stained with Massons trichrome. Original magnification x 400, bar = 25μm.

Figure 3. Periosteal bone formation rates throughout the adult skeleton. Untreated adult female cynomolgus monkeys (n=18) were injected with calcein three months apart and formation rates were calculated at the radius mid-diaphysis, femoral neck, femoral mid-diaphysis, 2^{nd} lumbar vertebra, and humeral mid-diaphysis. Data presented as mean ± SE. Overall ANOVA p values = 0.04. See R. Brommage et al. J Clin Endocrinol Metab. 1999 for further study information.

References

1. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M, Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J, Watts NB (2003) Effects of Estrogen Plus Progestin on Risk of Fracture and Bone Mineral Density: The Women's Health Initiative Randomized Trial. JAMA 290:1729-1738
2. Cummings SR, Karpf DB, Harris F, Genant HK, Ensrud K, LaCroix AZ, Black DM (2002) Improvement in spine bone density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. Am J Med 112:281-289
3. Wasnich RD, Miller PD (2000) Antifracture Efficacy of Antiresorptive Agents Are Related to Changes in Bone Density. J Clin Endocrinol Metab 85:231-236
4. Crabtree N, Loveridge N, Parker M, Rushton N, Power J, Bell KL, Beck TJ, Reeve J (2001) Intracapsular hip fracture and the region-specific loss of cortical bone: analysis by peripheral quantitative computed tomography. J Bone Miner Res 16:1318-1328
5. Duan Y, Seeman E, Turner CH (2001) The biomechanical basis of vertebral body fragility in men and women. J Bone Miner Res 16:2276-2283
6. Kalender WA, Felsenberg D, Louis O, Lopez P, Klotz E, Osteaux M, Fraga J (1989) Reference values for trabecular and cortical vertebral bone density in single and dual-energy quantitative computed tomography. Eur J Radiol 9:75-80
7. Alhlborg H, Johnell O, Turner C, Rannevik G, Karlsson M (2003) Bone loss and bone size after menopause. N Engl J Med 349:327-334
8. Orwoll E (2003) Toward an expanded understanding of the role of the periosteum in skeletal health. J Bone Miner Res 18:949-954
9. Beck TJ, Ruff CB, Scott WW, Jr., Plato CC, Tobin JD, Quan CA (1992) Sex differences in geometry of the femoral neck with aging: a structural analysis of bone mineral data. Calcif Tissue Int 50:24-29
10. Jones DB, Nolte H, Scholubbers JG, Turner E, Veltel D (1991) Biochemical signal transduction of mechanical strain in osteoblast-like cells. Biomaterials 12:101-110
11. Midura RJ, Su X, Morcuende JA, Tammi M, Tammi R (2003) Parathyroid hormone rapidly stimulates hyaluronan synthesis by periosteal osteoblasts in the tibial diaphysis of the growing rat. J. Biol. Chem.:M307567200
12. Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF, Kudo A (1999) Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. J Bone Miner Res 14:1239-1249
13. Oshima A, Tanabe H, Yan T, Lowe GN, Glackin CA, Kudo A (2002) 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 86:792-804
14. Takeshita S, Kikuno R, Tezuka K, Amann E (1993) Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. Biochem J 294 (Pt 1):271-278
15. Bord S, Horner A, Beavan S, Compston J (2001) Estrogen receptors alpha and beta are differentially expressed in developing human bone. J Clin Endocrinol Metab

86:2309-2314
16. Epker BN, Frost HM (1965) A histological study of remodeling at the periosteal, haversian canal, cortical endosteal, and trabecular endosteal surfaces in human rib. Anat Rec 152:129-135
17. Frost HM (1973) Bone modeling and skeletel modeling errors. Thomas, Springfield, IL
18. Ferretti JL, Frost HM, Gasser JA, High WB, Jee WS, Jerome C, Mosekilde L, Thompson DD (1995) Perspectives on osteoporosis research: its focus and some insights from a new paradigm. Calcif Tissue Int 57:399-404
19. Jee WS (2001) Integrated Bone Tissue Physiology: Anatomy and Physiology. In: Cowin S (ed) Bone Mechanics Handbook. CRC Press, Boca Raton
20. Pankovich A (1975) Primary internal fixation of femoral neck fractures. Arch Surg 110:20-26
21. Phemister D (1939) The pathology of ununited fractures of the neck of the femur with special reference to the head. J Bone Joint Surg Am 21:681-693
22. Banks H (1964) Healing of the femoral neck fracture. Proceedings of the Conference on Aseptic Necrosis of the Femoral Head:465-482
23. Bagi CM, Wilkie D, Georgelos K, Williams D, Bertolini D (1997) Morphological and structural characteristics of the proximal femur in human and rat. Bone 21:261-267
24. Power J, Loveridge N, Rushton N, Parker M, Reeve J (2003) Evidence for bone formation on the external "periosteal" surface of the femoral neck: a comparison of intracapsular hip fracture cases and controls. Osteoporos Int 14:141-145
25. Shea JE, Hallows RK, Ricks S, Bloebaum RD (2002) Microvascularization of the hypermineralized calcified fibrocartilage and cortical bone in the sheep proximal femur. Anat Rec 268:365-370
26. Shea JE, Vajda EG, Bloebaum RD (2001) Evidence of a hypermineralised calcified fibrocartilage on the human femoral neck and lesser trochanter. J Anat 198:153-162
27. Dixon T, Benjamin J, Lund P, Graham A, Krupinski E (2000) Femoral neck buttressing: a radiographic and histologic analysis. Skeletal Radiol 29:587-592
28. Chyun Y, Kream B, Raisz L (1984) Cortisol decreases bone formation by inhibiting periosteal cell proliferation. Endocrinology 114:477-480
29. Bianco P, Riminucci M, Gronthos S, Robey PG (2001) Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 19:180-192
30. Raisz L (1984) Studies on bone formation and resorption in vitro. Hormone Research 20:22-27
31. Hall B (ed) (1992) Bone Growth. CRC Press, Boca Raton
32. Ellender G, Feik SA, Carach BJ (1988) Periosteal structure and development in a rat caudal vertebra. J Anat 158:173-187
33. Hohmann EL, Elde RP, Rysavy JA, Einzig S, Gebhard RL (1986) Inervation of periosteum and bone by sympathetic vasoactive intestinal peptide-containing nerve fibers. Science 232:868
34. Aubin J, Triffitt J (2002) Mesenchymal stem cells and osteoblast differentiation. In: Bilezikian J, Raisz LG, Rodan GA (eds) Principles of Bone Biology. Academic Press, San Diego
35. Squier C, Ghoneim S, Kremenak C (1990) Ultrastructure of the periosteum from membrane bone. J Anat 171:233-239

36. Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, Keyser CP, Clohisy DR, Adams DJ, O'Leary P, Mantyh PW (2002) Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. Neuroscience 113:155-166
37. Tonna EA, Cronkite EP (1963) The periosteum. Autoradiographic studies on cellular proliferation and transformation utilizing tritiated thymidine. Clin Orthop 30:218-233
38. O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS (2001) The chondrogenic potential of periosteum decreases with age. J Orthop Res 19:95-103
39. Nakahara H, Goldberg VM, Caplan AI (1991) Culture-expanded human periosteal-derived cells exhibit osteochondral potential in vivo. J Orthop Res 9:465-476
40. Epker BN, Frost HM (1966) Periosteal appositional bone growth from age two to age seventy in man. A tetracycline evaluation. Anat Rec 154:573-577
41. Pfeilschifter J, Diel I, Pilz U, Brunotte K, Naumann A, Ziegler R (1993) Mitogenic responsiveness of human bone cells in vitro to hormones and growth factors decreases with age. J Bone Miner Res 8:707-717
42. Kim BT, Mosekilde L, Duan Y, Zhang XZ, Tornvig L, Thomsen JS, Seeman E (2003) The structural and hormonal basis of sex differences in peak appendicular bone strength in rats. J Bone Miner Res 18:150-155
43. Diaz-Flores L, Gutierrez R, Lopez-Alonso A, Gonzalez R, Varela H (1992) Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. Clin Orthop:280-286
44. Reilly TM, Seldes R, Luchetti W, Brighton CT (1998) Similarities in the phenotypic expression of pericytes and bone cells. Clin Orthop:95-103
45. Brighton CT, Lorich DG, Kupcha R, Reilly TM, Jones AR, Woodbury RA, 2nd (1992) The pericyte as a possible osteoblast progenitor cell. Clin Orthop:287-299
46. Beck TJ, Ruff CB, Bissessur K (1993) Age-related changes in female femoral neck geometry: implications for bone strength. Calcif Tissue Int 53 Suppl 1:S41-46
47. Ruff CB, Hayes WC (1982) Subperiosteal expansion and cortical remodeling of the human femur and tibia with aging. Science 217:945-948
48. Anderson C, Danylchuk K (1978) Bone-remodeling rates of the beagle: a comparison between different sites on the same rib. Am J Vet Res 39:1763-1765
49. Miller SC, Bowman BM, Miller MA, Bagi CM (1991) Calcium absorption and osseous organ-, tissue-, and envelope-specific changes following ovariectomy in rats. Bone 12:439-446
50. Sheng M, Baylink D, Beamer W, Donahue L, Rosen C, Lau K, Wergedal J (1999) Histmorphometric 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 25:421-429
51. Hock JM, Kream BE, Raisz LG (1982) Autoradiographic study of the effect of 1,25-dihydroxyvitamin D3 on bone matrix synthesis in vitamin D replete rats. Calcif Tissue Int 34:347-351
52. Leis HJ, Hulla W, Gruber R, Huber E, Zach D, Gleispach H, Windischhofer W (1997) Phenotypic heterogeneity of osteoblast-like MC3T3-E1 cells: changes of bradykinin-induced prostaglandin E2 production during osteoblast maturation. J Bone Miner Res 12:541-551
53. Solchaga LA, Cassiede P, Caplan AI (1998) Different response to osteo-inductive agents in bone marrow- and periosteum-derived cell preparations. Acta Orthop

Scand 69:426-432
54. Dempster D, Zhou H, Cosman F, Nieves J, Adachi J, Frahar L, Watson P, Lindsay B, Hodsman A (2001) PTH treatment directly stimulates bone formation in cancellous and cortical bone in humans. J Bone Miner Res 16 (Supp 1):S179
55. Mashiba T, Burr DB, Turner CH, Sato M, Cain RL, Hock JM (2001) Effects of human parathyroid hormone (1-34), LY333334, on bone mass, remodeling, and mechanical properties of cortical bone during the first remodeling cycle in rabbits. Bone 28:538-547
56. Bolotin H, Sievanen H (2001) Inaccuracies inherent in dual-energy x-ray absorptiometry in vivo bone mineral density can seriously mislead diagnostic/prognostic interpretations of patient-specific bone fragility. J Bone Miner Res 16:799
57. Burr D, Hirano T, Turner C, Hotchkiss C, Brommage R, Hock J (2001) Intermittently administered human parathyroid hormone(1-34) treatment increases intracortical bone turnover and porosity without reducing bone strength in the humerus of ovariectomized cynomolgus monkeys. Journal of Bone and Mineral Research 16:157-165
58. Parfitt A (1994) Osteonal and hemi-osteonal remodeling: the spatial and temporal framework for signal traffic in adult human bone. J. Cellular Biochemistry 55:273-286
59. Parfitt AM (2002) Parathyroid hormone and periosteal bone expansion. J Bone Miner Res 17:1741-1743
60. Mohan S, Kutilek S, Zhang C, Shen H, Y. K, Srivastava A (2000) Comparison of bone formation responses to parathyroid hormone (1-34), (1-31), and (2-34) in mice. Bone 27:471-478
61. Rehman Q, Lang TF, Arnaud CD, Modin GW, Lane NE (2003) Daily treatment with parathyroid hormone is associated with an increase in vertebral cross-sectional area in postmenopausal women with glucocorticoid-induced osteoporosis. Osteoporos Int 14:77-81
62. Zanchetta JR, Bogado CE, Ferretti JL, Wang O, Wilson MG, Sato M, Gaich GA, Dalsky GP, Myers SL (2003) Effects of teriparatide [recombinant human parathyroid hormone (1-34)] on cortical bone in postmenopausal women with osteoporosis. J Bone Miner Res 18:539-543
63. Dempster DW, Cosman F, Kurland ES, Zhou H, Nieves J, Woelfert L, Shane E, Plavetic K, Muller R, Bilezikian J, Lindsay R (2001) Effects of daily treatment with parathyroid hormone on bone microarchitecture and turnover in patients with osteoporosis: a paired biopsy study. J Bone Miner Res 16:1846-1853
64. Hodsman AB, Kisiel M, Adachi JD, Fraher LJ, Watson PH (2000) Histomorphometric evidence for increased bone turnover without change in cortical thickness or porosity after 2 years of cyclical hPTH(1-34) therapy in women with severe osteoporosis. Bone 27:311-318
65. Jiang Y, Zhao J, Mitlak B, Wang O, Genant H, Eriksen E (2003) Recombinant human parathyroid hormone (1-34) [teriparatide] improves both cortical and cancellous bone structure. J Bone Miner Res 18:1932-1941
66. Black DM, Greenspan SL, Ensrud KE, Palermo L, McGowan JA, Lang TF, Garnero P, Bouxsein ML, Bilezikian JP, Rosen CJ (2003) The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. N Engl J

Med 349:1207-1215
67. Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM (2003) The effects of parathyroid hormone, alendronate, or both in men with osteoporosis. N Engl J Med 349:1216-1226
68. Ettinger B, San Martin J, Crans G, Pavo I (2004) Differential effects of teriparatide on BMD after treatment with raloxifene or alendronate. J Bone Miner Res 19:745-751
69. Delmas PD, Vergnaud P, Arlot ME, Pastoureau P, Meunier PJ, Nilssen MH (1995) The anabolic effect of human PTH (1-34) on bone formation is blunted when bone resorption is inhibited by the bisphosphonate tiludronate--is activated resorption a prerequisite for the in vivo effect of PTH on formation in a remodeling system? Bone 16:603-610
70. Ma YL, Bryant HU, Zeng Q, Schmidt A, Hoover J, Cole HW, Yao W, Jee WS, Sato M (2003) New bone formation with teriparatide [human parathyroid hormone-(1-34)] is not retarded by long-term pretreatment with alendronate, estrogen, or raloxifene in ovariectomized rats. Endocrinology 144:2008-2015
71. Hock JM, Hummert JR, Boyce R, Fonseca J, Raisz LG (1989) Resorption is not essential for the stimulation of bone growth by hPTH-(1-34) in rats in vivo. J Bone Miner Res 4:449-458
72. Hock JM (2000) Discrimination among osteoblasts? Parathyroid hormone analog may reveal site-specific differences in mice. Bone 27:467-469
73. Morinaga T, Nakagawa N, Yasuda H, Tsuda E, Higashio K (1998) Cloning and characterization of the gene encoding human osteoprotegerin/osteoclastogenesis-inhibitory factor. Eur J Biochem 254:685-691
74. Christiansen P, Steiniche T, Brixen K, Hessov I, Melsen F, Heickendorff L, Mosekilde L (1999) Primary hyperparathyroidism: short-term changes in bone remodeling and bone mineral density following parathyroidectomy. Bone 25:237-244
75. Christiansen P, Steiniche T, Mosekilde L, Hessov I, Melsen F (1990) Primary hyperparathyroidism: changes in trabecular bone remodeling following surgical treatment--evaluated by histomorphometric methods. Bone 11:75-79
76. Christiansen P, Steiniche T, Vesterby A, Mosekilde L, Hessov I, Melsen F (1992) Primary hyperparathyroidism: iliac crest trabecular bone volume, structure, remodeling, and balance evaluated by histomorphometric methods. Bone 13:41-49
77. Dobnig H, Turner RT (1997) The Effects of Programmed Administration of Human Parathyroid Hormone Fragment (1-34) on Bone Histomorphometry and Serum Chemistry in Rats. Endocrinology 138:4607-4612
78. Jaeger P, Jones W, Kashgarian M, Baron R, Clemens T, Segre G, Hayslett J (1987) Animal model of primary hyperparathyroidism. Am J Physiol 252:E790-798
79. Imanishi Y, Hosokawa Y, Yoshimoto K, Schipani E, Mallya S, Papanikolaou A, Kifor O, Tokura T, Sablosky M, Ledgard F, Gronowicz G, Wang TC, Schmidt EV, Hall C, Brown EM, Bronson R, Arnold A (2001) Primary hyperparathyroidism caused by parathyroid-targeted overexpression of cyclin D1 in transgenic mice. J. Clin. Invest. 107:1093-1102
80. Calvi LM, Sims NA, Hunzelman JL, Knight MC, Giovannetti A, Saxton JM, Kronenberg HM, Baron R, Schipani E (2001) Activated parathyroid hormone/parathyroid hormone-related protein receptor in osteoblastic cells

differentially affects cortical and trabecular bone. J Clin Invest 107:277-286
81. Harris WH, Heaney RP, Jowsey J, Cockin J, Akins C, Graham J, Weinberg EH (1972) Growth hormone: the effect on skeletal renewal in the adult dog. I. Morphometric studies. Calcif Tissue Res 10:1-13
82. Andreassen TT, Melsen F, Oxlund H (1996) The influence of growth hormone on cancellous and cortical bone of the vertebral body in aged rats. J Bone Miner Res 11:1094-1102
83. Andreassen TT, Oxlund H (2000) The influence of combined parathyroid hormone and growth hormone treatment on cortical bone in aged ovariectomized rats. J Bone Miner Res 15:2266-2275
84. Kruse HP, Kuhlencordt F (1975) On an attempt to treat primary and secondary osteoporosis with human growth hormone. Horm Metab Res 7:488-491
85. Landin-Wilhelmsen K, Nilsson A, Bosaeus I, Bengtsson BA (2003) Growth hormone increases bone mineral content in postmenopausal osteoporosis: a randomized placebo-controlled trial. J Bone Miner Res 18:393-405
86. Mohan S, Richman C, Guo R, Amaar Y, Donahue LR, Wergedal J, Baylink DJ (2003) Insulin-Like Growth Factor Regulates Peak Bone Mineral Density in Mice by Both Growth Hormone-Dependent and -Independent Mechanisms. Endocrinology 144:929-936
87. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT, Setser J, Frystyk J, Boisclair YR, LeRoith D (2002) Circulating levels of IGF-1 directly regulate bone growth and density. J Clin Invest 110:771-781
88. Tobias JH, Chow JW, Chambers TJ (1992) Opposite effects of insulin-like growth factor-I on the formation of trabecular and cortical bone in adult female rats. Endocrinology 131:2387-2392
89. Ghiron LJ, Thompson JL, Holloway L, Hintz RL, Butterfield GE, Hoffman AR, Marcus R (1995) Effects of recombinant insulin-like growth factor-I and growth hormone on bone turnover in elderly women. J Bone Miner Res 10:1844-1852
90. Rosen CJ, Wuster C (2003) Growth hormone rising: did we quit too quickly? J Bone Miner Res 18:406-409
91. Mashiba T, Hirano T, Turner CH, Forwood MR, Johnston CC, Burr DB (2000) Suppressed bone turnover by bisphosphonates increases microdamage accumulation and reduces some biomechanical properties in dog rib. J Bone Miner Res 15:613-620
92. Itoh F, Kojima M, Furihata-Komatsu H, Aoyagi S, Kusama H, Komatsu H, Nakamura T (2002) Reductions in bone mass, structure, and strength in axial and appendicular skeletons associated with increased turnover after ovariectomy in mature cynomolgus monkeys and preventive effects of clodronate. J Bone Miner Res 17:534-543
93. Dufresne T, Chmielewski P, Manhart M, Johnson T, Borah B (2003) Risedronate Preserves Bone Architecture in Early Postmenopausal Women In 1 Year as Measured by Three-Dimensional Microcomputed Tomography. Calcif Tissue Int 73:423-432
94. Giuliani N, Pedrazzoni M, Negri G, Passeri G, Impicciatore M, Girasole G (1998) Bisphosphonates stimulate formation of osteoblast precursors and mineralized nodules in murine and human bone marrow cultures in vitro and promote early osteoblastogenesis in young and aged mice in vivo. Bone 22:455-461

95. Goziotis A, Sukhu B, Torontali M, Dowhaniuk M, Tenenbaum HC (1995) Effects of bisphosphonates APD and HEBP on bone metabolism in vitro. Bone 16:317S-327S
96. Im GI, Qureshi SA, Kenney J, Rubash HE, Shanbhag AS (2004) Osteoblast proliferation and maturation by bisphosphonates. Biomaterials 25:4105-4115
97. Balena R, Shih MS, Parfitt AM (1992) Bone resorption and formation on the periosteal envelope of the ilium: a histomorphometric study in healthy women. J Bone Miner Res 7:1475-1482
98. Plotkin LI, Manolagas SC, Bellido T (2002) Transduction of cell survival signals by connexin-43 hemichannels. J Biol Chem 277:8648-8657
99. Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T (1999) Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. J Clin Invest 104:1363-1374
100. Turner RT, Riggs BL, Spelsberg TC (1994) Skeletal effects of estrogen. Endocr Rev 15:275-300
101. Turner RT, Hannon KS, Demers LM, Buchanan J, Bell NH (1989) Differential effects of gonadal function on bone histomorphometry in male and female rats. J Bone Miner Res 4:557-563
102. Wakley GK, Evans GL, Turner RT (1997) Short-term effects of high dose estrogen on tibiae of growing male rats. Calcif Tissue Int 60:37-42
103. Coxam V, Bowman BM, Mecham M, Roth CM, Miller MA, Miller SC (1996) Effects of dihydrotestosterone alone and combined with estrogen on bone mineral density, bone growth, and formation rates in ovariectomized rats. Bone 19:107-114
104. Gunness M, Orwoll E (1995) Early induction of alterations in cancellous and cortical bone histology after orchiectomy in mature rats. J Bone Miner Res 10:1735-1744
105. Lea C, Kendall N, Flanagan AM (1996) Casodex (a nonsteroidal antiandrogen) reduces cancellous, endosteal, and periosteal bone formation in estrogen-replete female rats. Calcif Tissue Int 58:268-272
106. Kasperk C, Helmboldt A, Borcsok I, Heuthe S, Cloos O, Niethard F, Ziegler R (1997) Skeletal site-dependent expression of the androgen receptor in human osteoblastic cell populations. Calcif Tissue Int 61:464-473
107. Shozu M, Simpson ER (1998) Aromatase expression of human osteoblast-like cells. Mol Cell Endocrinol 139:117-129
108. Turner RT, Bleiberg B, Colvard DS, Keeting PE, Evans G, Spelsberg TC (1990) Failure of isolated rat tibial periosteal cells to 5 alpha reduce testosterone to 5 alpha-dihydrotestosterone. J Bone Miner Res 5:775-779
109. Vidal O, Lindberg MK, Hollberg K, Baylink DJ, Andersson G, Lubahn DB, Mohan S, Gustafsson J-A, Ohlsson C (2000) Estrogen receptor specificity in the regulation of skeletal growth and maturation in male mice. Proceedings of the National Academy of Sciences 97:5474-5479
110. Sims NA, Dupont S, Krust A, Clement-Lacroix P, Minet D, Resche-Rigon M, Gaillard-Kelly M, Baron R (2002) Deletion of estrogen receptors reveals a regulatory role for estrogen receptors-[beta] in bone remodeling in females but not in males. Bone 30:18-25
111. Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L (2003) Endocrinology: bone adaptation requires oestrogen receptor-alpha. Nature 424:389
112. Jessop H, Suswillow R, Rawlinson S, Zaman G, Lee K, Das-Gupta V, Pitsillides A, Lanyon L (2004) Osteoblast-like cells from estrogen receptor [alpha] knockout mice

have deficient responses to mechanical strain. J Bone Miner Res 19:938-946

113. Vandenput L, Swinnen J, SBoonen S, Van Herck E, Erben R, Bouillion R, Vanderschueren D Role of the androgen receptor in skeletal homeostasis: the androgen-resistant testicular feminized male mouse model. J Bone Miner Res Published online May 10, 2004; doi: 10.1359/JBMR.040505

114. Wiren K, Zhang X, Toombs A, Kasparcova V, Gentile M, Harada S, Jepsen K Targeted overexpression of androgen receptor in osteoblasts: unexpected complex bone phenotype in growing animals. Endocrinology Published online May 6, 2004; doi: 10.1210/en.2003-1016

115. Seeman E (2003) The structural and biomechanical basis of the gain and loss of bone strength in women and men. Endocrinol Metab Clin North Am 32:25-38

116. Seeman E (2001) Clinical review 137: Sexual dimorphism in skeletal size, density, and strength. J Clin Endocrinol Metab 86:4576-4584

117. Vedi S, Bell KL, Loveridge N, Garrahan N, Purdie DW, Compston JE (2003) The effects of hormone replacement therapy on cortical bone in postmenopausal women. A histomorphometric study. Bone 33:330-334

118. Beck TJ, Stone KL, Oreskovic TL, Hochberg MC, Nevitt MC, Genant HK, Cummings SR (2001) Effects of current and discontinued estrogen replacement therapy on hip structural geometry: the study of osteoporotic fractures. J Bone Miner Res 16:2103-2110

119. Ott SM, Oleksik A, Lu Y, Harper K, Lips P (2002) Bone histomorphometric and biochemical marker results of a 2-year placebo-controlled trial of raloxifene in postmenopausal women. J Bone Miner Res 17:341-348

120. Lazenby RA (1990) Continuing periosteal apposition. I: Documentation, hypotheses, and interpretation. Am J Phys Anthropol 82:451-472

121. Seeman E, Duan Y (2004) Measurement Issues in Periosteal Apposition. J Bone Miner Res 19:691

122. Masinde GL, Wergedal J, Davidson H, Mohan S, Li R, Li X, Baylink DJ (2003) Quantitative trait loci for periosteal circumference (PC): identification of single loci and epistatic effects in F2 MRL/SJL mice. Bone 32:554-560

123. Nakazawa T, Nakajima A, Seki N, Okawa A, Kato M, Moriya H, Amizuka N, Einhorn TA, Yamazaki M (2004) Gene expression of periostin in the early stage of fracture healing detected by cDNA microarray analysis. J Orthop Res 22:520-525

124. Seeman E (2003) Periosteal bone formation--a neglected determinant of bone strength. N Engl J Med 349:320-323

125. Seeman E (2003) Reduced bone formation and increased bone resorption: rational targets for the treatment of osteoporosis. Osteoporos Int 14 Suppl 3:S2-8

126. Seeman E (2002) An exercise in geometry. J Bone Miner Res 17:373-380

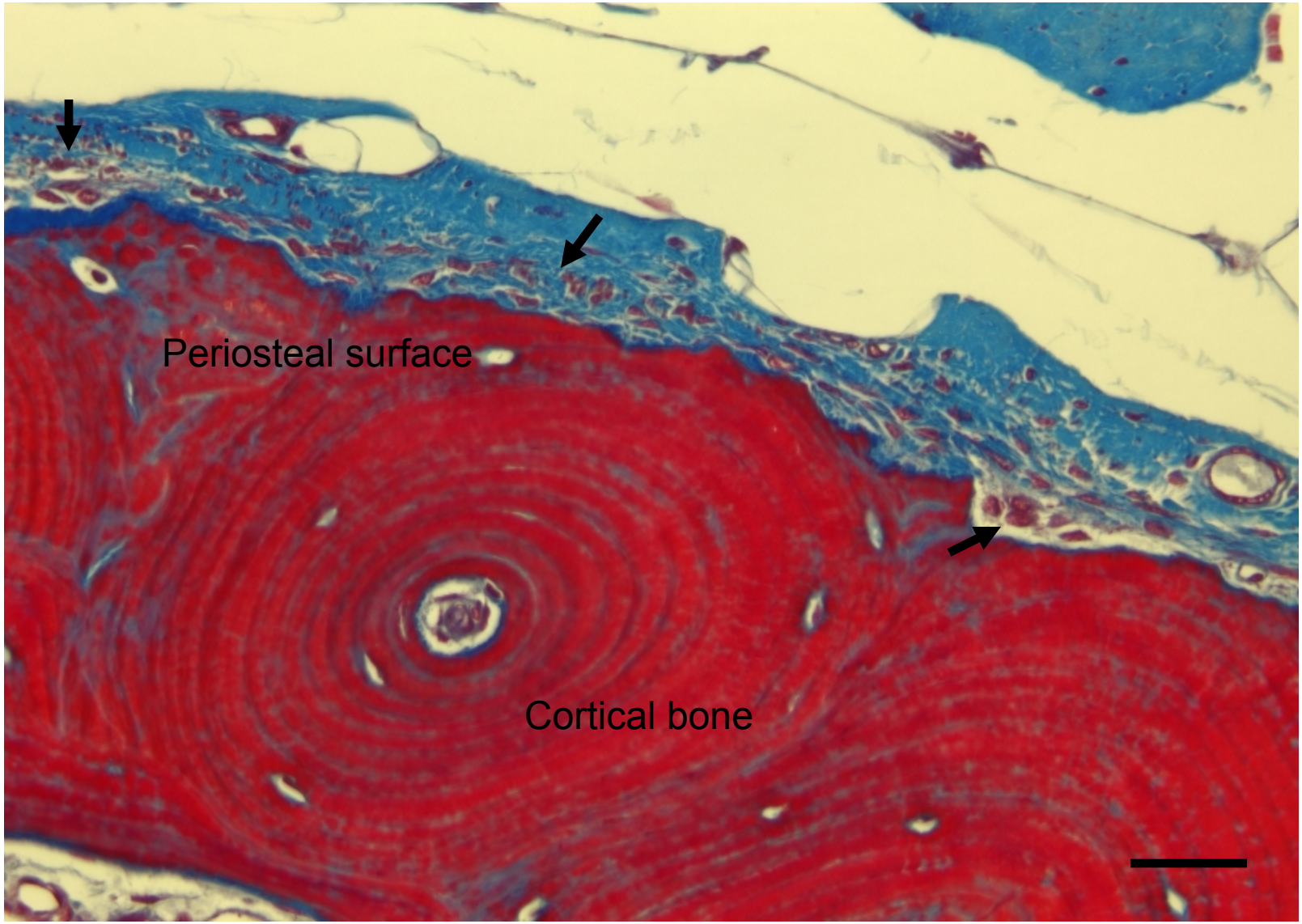


Figure 1

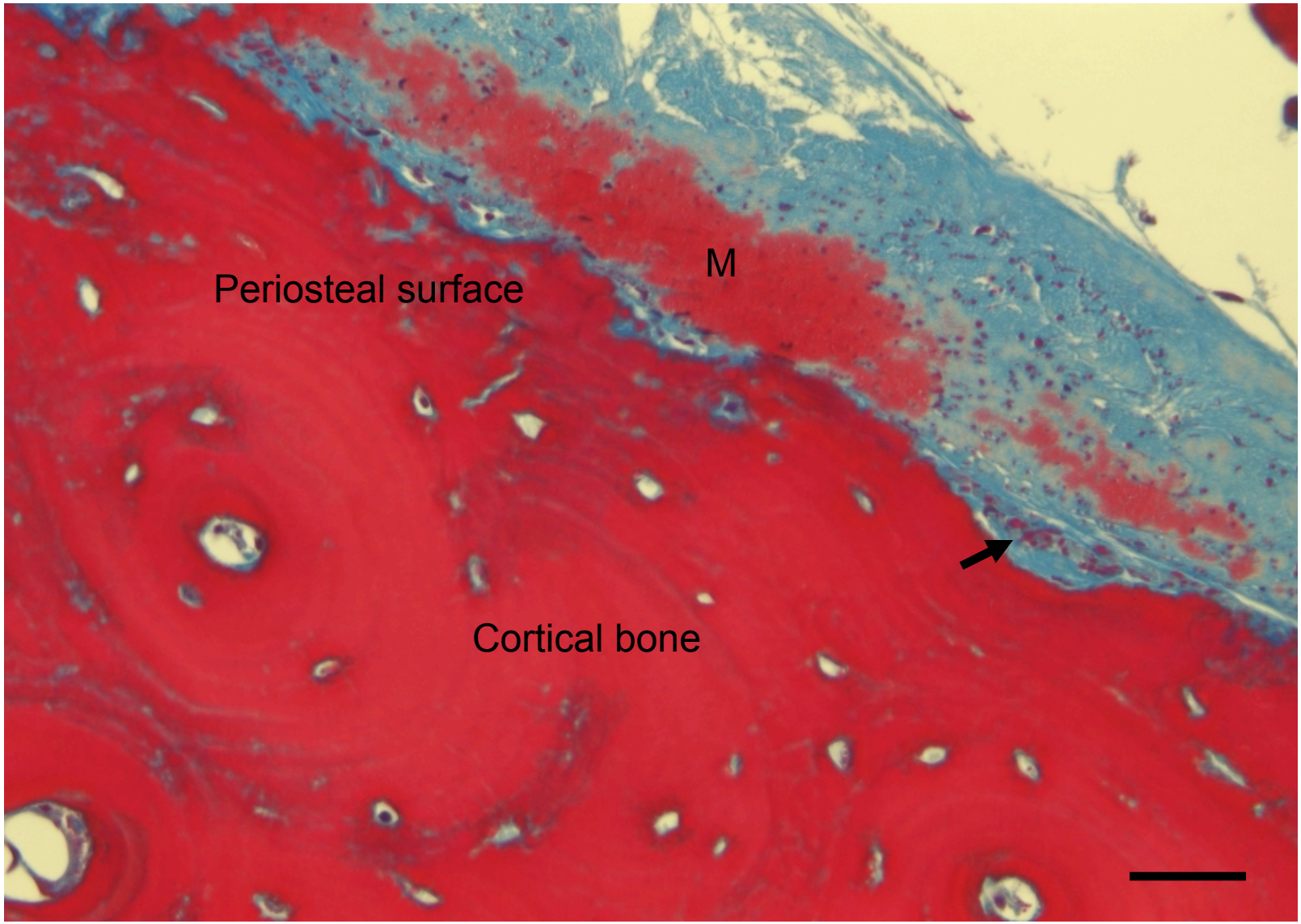
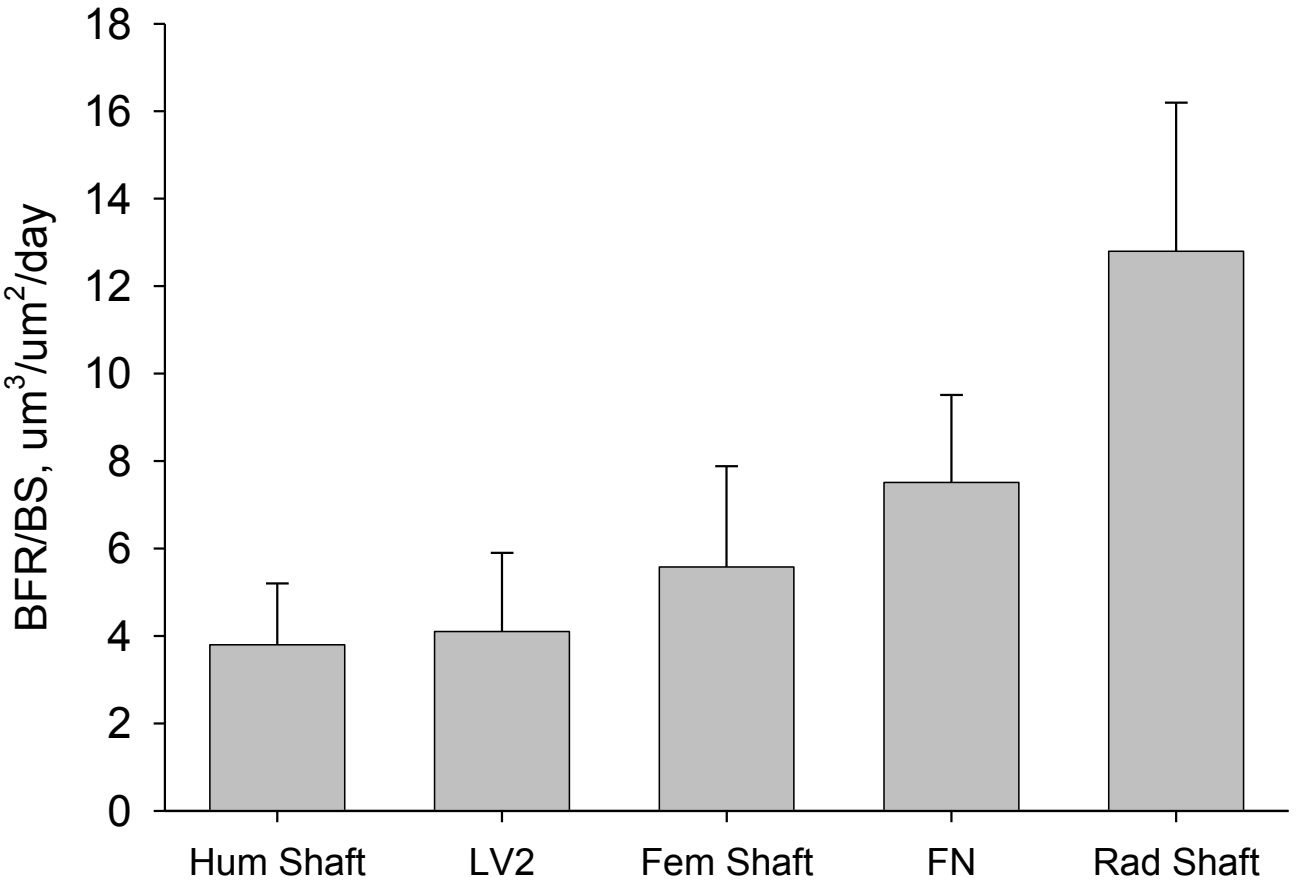


Figure 2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Rad Shaft

Figure 3