J Musculoskelet Neuronal Interact 2006; 6(2):128-133

Perspective Article

Hylonome

Coupled or uncoupled remodeling, is that the question?

J.A. Gasser

Novartis Institutes for Biomedical Research, Basel, Switzerland

Keywords: Bone Remodelling, Coupling, Parathyroid Hormone, Mechanosensing

The American Daniel Boorstin was a powerful and original social historian and critic. He taught at the University of Chicago for 25 years, and served as librarian of the US Congress for 12 years. He published more than 20 books, among them The Discoverers (1983) on scientists and inventors. It is out of this book that I have taken the quote, which in my opinion, so adequately describes the situation in science and research: "The great obstacle to progress is not ignorance, but the illusion of knowledge". Very often dogmas are generated out of our "illusion of knowledge" ending up as "common knowledge" in the minds of people and in textbooks that may remain unchallenged for decades. In addition dogmas may impose a bias on the interpretation of new data since most scientists may attempt to fit their findings into existing models rather than trying to challenge them.

The things we believe we know or the 'known knowns'

The widely held belief that bone resorption is always coupled to bone formation in a process called 'bone remodeling cycle' first described by Harold Frost[1], may be such a dogma. According to commonly held beliefs, bone resorption and the recruitment of osteoclasts, which derive from the mononuclear/phagocytic lineage, to the remodeling site are under the control of factors secreted by osteoblasts[2,3].

Bone resorption

There is no doubt that osteoblasts express cytokines in their membrane, and in particular, colony-stimulating factor 1 (CSF-1) and RANKL, which can be cleaved to activate osteoclastogenesis in a local, paracrine manner. They also secrete osteoprotegerin, a decoy RANK receptor capable of inhibiting osteoclast formation. The extracellular bone-resorbing compartment generated by the osteoclast is the functional equivalent of a secondary lysosome, with a low pH, lysosomal enzymes and the substrate, the bone matrix[4]. First, the hydroxyapatite crystals are mobilized by digestion of their link to collagen (the non-collagenous proteins) and dissolved by the acid environment. Then, the residual collagen fibers are digested either by the activation of latent collagenase or by the action of cathepsins at low pH which are secreted through the ruffled border into the extracellular bone-resorbing compartment. Furthermore, the cell secretes several metalloproteases such as collagenase and gelatinase. They reach a sufficiently high extracellular concentration because this compartment is sealed off. The enzymes, now at optimal pH, degrade the matrix components; the residues from this extracellular digestion are internalized, transported across the cell (by transcytosis) and released at the basolateral domain, or released during periods of relapse of the sealing zone, possibly induced by a calcium sensor responding to the rise of extracellular calcium in the bone-resorbing compartment.

Coupling of resorption to formation

It is widely believed that during the resorption process, growth factors like TGF[5,6], structural proteins such as type I collagens, osteocalcin or their fragments[7,8] liberated from the bone matrix are responsible for the activation of bone formation (coupling of resorption to formation) since there is *in vitro* evidence that they can act as chemoattractants for osteoblasts. Consequently these factors are proposed to activate the process of bone formation after completion of the reversal phase, which represents the last step in the remodeling cycle. Likewise, based on *in vitro* experiments, the differentiation of mesenchymal precursor cells to mature osteoblasts, and osteoblast proliferation were proposed to be under the control of growth factors such as TGFI and II, BMPs, PDGF, IGFI and II and FGF[9,10] which are released

The author has a corporate appointment with Novartis.

Corresponding author: Jürg A. Gasser, Ph.D., Novartis Institutes for Biomedical Research, Musculoskeletal Diseases, WKL-125.10.15, Basel CH-4002, Switzerland
E-mail: juerg.gasser@novartis.com

Accepted 30 January 2006

locally as a consequence of the bone resorption process from the bone matrix.

There is not much information in the literature about the regulation of the final phase of bone formation, the cessation of osteoblast function. One proposal is that active TGF decreases the function of mature osteoblasts[9].

The 'known unknowns' in bone remodeling?

In vitro studies have provided a tremendous amount of data that has been interpreted to generate the ARF-model (Activation, Resorption, Formation) by Frost on the regulation of bone remodeling. Despite these efforts many questions have not been addressed or in some cases, the current beliefs can be easily challenged. Below are some important questions for which no clear answer has been found to date in the ARF-model. Addressing them appears to be of fundamental importance if we wish to truly understand the complex regulation of this process.

Activation

1. Which mechanism determines where remodeling occurs?
2. Who or what initiates retraction of lining cells at the site of remodelling?

Resorption

1. Who determines that there is a need to recruit osteoclast precursor cells and induce their fusion to multinuclear OCs?
2. Who activates the bone resorption process and who stops it?

Formation

1. Who recruits osteoblasts to the resorption site?
2. Who controls their activity and determines when the remodeling cycle is complete?

One of the problems in accepting the current dogma on the regulation of the bone remodeling cycle is that in reality, bone resorption and bone formation processes are separated in space and time. In other words there are no apparent active osteoblasts on or adjacent to the 'future' remodeling site to cause the retraction of bone lining cells or recruit osteoclasts to initiate bone resorption, a process which requires approximately 8 days[11]. Likewise, it is difficult to understand that the matrix bound growth factors released during the 34-day resorption-phase should trigger osteoblast -mediated bone formation only after an additional 9 days of a quiescent period (reversal phase) and then continue to be active for approximately 130 days after bone resorption has ceased to induce osteoblast differentiation and proliferation[11]. Most currently accepted models of coupling of bone resorption and formation fail to explain why the formation process is stopped just when the resorption cycle is complete[11]. It is even more puzzling to explain why the resorption drifts at the periosteum in the lower metaphysis of long bones or the endocortical resorption drifts in the mid-diaphysis responsible for growth and shaping of long bones can continue for years without triggering the proposed growth factor-related coupling mechanism[12].

Last but not least, it is difficult to understand how growth factors like TGFs, PDGF, IGFs and FGFs released by the resorption process could survive the hostile acidic environment in the tightly sealed zone underneath the osteoclast without suffering degradation into inactive fragments by the protease rich environment, to be eventually released in the 'open space' at the basolateral surface of the osteoclast, where they could reach their target, either marrow stromal cells or mature osteoblasts after a journey of some distance through the bone marrow to the active site of bone formation.

A possible reconciliation?

There appears to be increasing agreement that the signal for induction of remodeling and the choice of site may be determined by the cells living within the matrix (osteocytes) which may sense microdamage or send out apoptotic signals[13-15]. Apart from this targeted remodeling, many authors have proposed that a proportion of bone remodeling which is involved in mineral balance is stochastic, that is to say not site-dependent[16,17], even though it is probably not an entirely random event.

Osteocytes are the terminal differentiation stage of the osteoblast, which when isolated directly from bone tissue, retain their morphologic feature *in vitro*[18-20]. Within the mineralized matrix they are in direct communication with each other and surface osteoblasts through their cellular processes which form a continuum by connection at the tip of their cell processes through gap junctions[21] formed by connexins, mainly connexin 43[22]. The primary function of the osteoblast (or lining cell)-osteocyte continuum is considered to be mechanosensory, by transducing stress signals (stretching, bending) sensed by the matrix embedded osteocytes to biological activity at the bone surface. The flow of extracellular fluid in response to mechanical forces throughout the canalicular system induces a spectrum of cellular responses in osteocytes[18]. Rapid fluxes of calcium across these junctions are thought to facilitate transmission of information between osteocytes within the bone structure and osteoblasts on the bone surface[23,24]. Signaling pathways mediating the mechanotransducing properties of osteocytes from the extracellular matrix through the cytoskeleton and finally to the nucleus to modulate gene transcription are beginning to be characterized. They involve prostaglandin (PGE_2) and cyclo-oxygenase 2, kinases, Cbfa1/Runx2, and nitrous oxide-mediated responsiveness of the bone to systemic factors and mechanical forces[25,26].

Osteocytes, due to their location in the load transmitting compartment, namely the mineralized matrix, are much better suited to direct the 'targeted' part of bone remodeling according to mechanical requirements, to detect disruption

of gap junctional message traffic by microdamage, and to provide the apoptotic signal and thus direct the repair response to the affected area than any other cell in bone[27,28]. Osteocytes would be a suitable candidate which may determine when and where remodeling has to occur, provide the necessary signal for retraction of the bone lining cells, provide the signal for the generation of osteoclasts and their homing to the desired remodeling site, and even to stop bone resorption, initiate the reversal phase, activation of osteoblast-mediated bone formation and detect when the remodeling is complete. The idea that BMU-coupling is regulated by strain is not new, but perhaps it did not receive the attention that it deserved[29]. The finite element model developed by Smit and Burger provides an interesting alternative explanation for the regulation of Haversian as well as surface based remodeling. Of course the question still remains, whether the 'stochastic' remodeling, which according to current thinking regulates mineral balance, is also guided by a tightly controlled process or whether it is truly random[16].

Part of the problem may have arisen from the fact that with the exception of chicken osteocytes, it is not possible to isolate these end-differentiated cells and study them *in vitro* over a long period of time in order to understand gap-junctional message traffic between them. It is also questionable if and to what degree an osteocyte cell culture based system would be able to simulate the complex interplay of the dynamically loaded osteocytic network with its connections to the surface based effector cells, the osteoblasts and osteoclasts, and its functioning feedback systems. On the other hand there appears to be little point in continuing all the intense work on the isolated osteoblast and osteoclast populations to find answers concerning the regulation of bone remodeling. It appears to be more logical to develop more complex *in vitro* systems instead where the complex interplay between sensor and effector cells is preserved better.

The case of PTH

The case of parathyroid hormone (PTH) shows how dogma-based thinking can affect the interpretation of results in a way that investigators try to fit their results into the existing dogma rather than trying to challenge it. In the case of PTH the existing dogma says that there is no modeling derived bone gain possible in the adult skeleton (after cessation of growth). As a consequence, it would follow that the strong bone anabolic effect of PTH must be explained by remodeling based bone gain or a positive bone balance at the level of the BMU.

PTH is a key factor in the control of 'stochastic' bone remodeling since it is the principal regulator of calcium homeostasis. Elevated levels of PTH increase bone turnover and lead to either anabolic or catabolic effects on the skeleton depending upon the pattern and duration of elevation. The principal target organs for PTH are the kidney where the hormone increases proximal tubular resorption of calcium, phosphate excretion and 1,25 dihydroxyvitamin D formation, and the skeleton.

Only 21 years after the arrival of the first data indicating that PTH was anabolic for bone, a randomized controlled sufficiently large (n=1,637) trial proved the anti-fracture efficacy of teriparatide in reducing both vertebral and non-vertebral fracture risk in osteoporotic women[30]. The reduction in risk of new vertebral fractures was 65% using once-daily 20μg injections, with an even greater risk reduction for moderate-to-severe fractures (90%). Secondary outcome measures in this and other trials of teriparatide (hPTH(1-34)) confirmed that BMD increased during 18 months of therapy at the lumbar spine and the femoral neck, but fell slightly at the radius. Nevertheless, the relative risk of wrist fractures in treated subjects was significantly lower than in controls[30]. BMD changes of a similar magnitude were observed in studies of men treated with teriparatide[31].

Histomorphometry has demonstrated improved trabecular thickness[32] and connectivity[33] in response to teriparatide, as well as evidence of periosteal and endosteal new bone formation[34,35]. Indeed, densitometry studies of treated patients confirmed (indirectly) that treatment increased subperiosteal bone formation in the distal radius[36] or cortical thickness in the femoral neck[37].

As mentioned above, the classical view about PTH-based bone gain is that the hormone acts by increasing mean wall thickness to create a positive bone balance in the remodeling cycle and the activation frequency leading to an increase in the number of events. This interpretation is upheld despite a lot of evidence that the majority of bone gain is likely to come from the activation of modeling drifts on virtually all bone surfaces, a process which many believe is only active during skeletal growth. In this author's opinion, the story of PTH represents a typical case where experimental evidence is forced into an existing dogma, even if it does not fit at all. Let's look at some of the arguments against remodeling based bone gain and those in support of activated modeling drifts to explain the powerful bone anabolic effects of PTH.

Bone gain following onset of PTH treatment is very rapid. In the study published by Orwoll BMD as measured by DEXA increased by 4% at 3 months and approximately 10% at 12 months[31]. This is very much in line with the findings by Lane and colleagues who found an increase in total spinal BMD of 12% at 1 year of treatment with a combination of PTH and estrogen using the same methodology. However, Lane and colleagues made an additional analysis of the cancellous bone compartment in the same vertebra by pQCT, which indicated a 35% increase in cancellous BMD[38]. It is not mathematically possible that the slow remodeling based bone gain which takes 6 months to complete the ARF-sequence could increase cancellous BMD by 35% in one year (two remodeling cycles). Interestingly, already the early studies by Hodsman showed that PTH administration results in a rapid increase in biochemical markers of bone formation, with a lesser and delayed increase in resorption markers[39]. The same response was seen following treatment with teriparatide[40]. To the same end, Orwoll found increased

bone ALP levels already at 1 month after starting PTH-treatment (first time point which was assessed) while NTX levels, an indicator of bone resorption gradually picked up peaking at 6 months only[31]. Bone gain with PTH appears to be highest during the first 6-12 months of treatment while bone gain slows down at the time when bone resorption (remodeling) is picking up. Hodsman and Steer[41] found histomorphometric evidence of osteoblast accumulation in response to teriparatide injections within six weeks (the first time point they investigated).

It seems increasingly likely that the mechanism of action of PTH is a coordinated one involving cells in addition to active osteoblasts, such as osteocytes and bone-lining cells[42]. De novo bone formation on previously quiescent surfaces ('renewed modelling') without evidence of cellular proliferation led Dobnig and Turner[43] to conclude that the lining cell could revert to an active osteoblast phenotype at the initiation of intermittent PTH therapy, views supported by histological evidence[41,44]. Recent evidence suggests that osteocytes rapidly downregulate sclerostin, a key osteoblast inhibitory protein, in response to intermittent PTH[45]. It is thus possible that the widespread osteocytic expression of sclerostin observed in human bone maintains lining cells in a quiescent state on bone surfaces[46]. A decrease in osteocytic sclerostin production in response to PTH would thus allow formation of new bone on previously quiescent surfaces (as lining cells revert to an active bone-forming state) i.e., the activation of a modeling drift.

In contrast to remodeling based bone gain, the activation of modeling drifts by PTH which mimics the pattern of bone gain observed during skeletal growth could easily explain a 35% increase in cancellous BMD observed in patients in one year while remodeling based bone gain could not.

Conclusions

The consequences of all these deliberations are several fold. It may be misleading to extrapolate the interpretation of data derived from experiments carried out on surrogate cells to the real situation. Even though the osteocyte is derived from the osteoblast lineage, its putative role as a mechanosensor and a cell important for maintenance of the mineralized matrix in which it is embedded is distinctly different from the function of the osteoblast, which is bone formation. While we can learn a lot from studying osteoblasts in their role as bone forming cells they can probably tell us little about the molecular events which really guide the process of tissue renewal and repair. Consequently it does not make much sense to study osteoblasts as a surrogate for the osteocyte simply because we have difficulties in studying the human osteocyte directly because it cannot be isolated and put in culture. Even if we could isolate and culture these cells for a short period of time, they could very likely give us only limited insight into the mechanism of gap-junctional communication since they are no longer exposed to the habitual dynamic changes in tissue strains in their natural environment, the mineralized matrix, and no longer connected to the complex feed-back systems which guide their activities. Bearing this in mind it would make more sense to step up the efforts to develop more complex 3D organ culture systems[47], or try to advance methods of non-invasive molecular imaging[48].

The PTH story is just a kind reminder that, while it is legitimate to compare new results with the evidence derived from older experiments to see whether they fit into existing models, this process should not be enforced at all cost. As scientists we have an obligation to look at our data with an open mind and if necessary, adjust our models and in some cases to have the courage to challenge some of the existing dogmas.

Let us not forget what T.H. Huxley said: "The known is finite, the unknown infinite; intellectually we stand on an islet in the midst of an illimitable ocean of inexplicability. Our business in every generation is to reclaim a little more land." It is with this spirit and an open mind that we should focus our research on the 'known unknowns' and sometimes even the apparently 'known knowns' to finally discover the real source of trouble, the 'unknown unknowns' or in other words, the things we don't know we don't know.

References

1. Frost HM. Dynamics of bone remodeling. In: Little and Brown (eds) BoneBiodynamics. Churchill **ISBN**: B0000CMFKJ, Boston, MA, USA; 1964:315.
2. Takahashi N, Udagawa N, Takami M, Suda T. Cells in bone, osteoclast generation. In: Bilezikian J (ed) Principles of Bone Biology, 2nd Vol 1, Academic Press, San Diego, CA, USA; 2002:109-126.
3. Boyle WJ, Simmonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423:337-342.
4. Teitelbaum SL. Bone resorption by osteoclasts. Science 2000; 289:1504-1508.
5. Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR, Boyce BF. Estrogen promotes apoptosis of murine octeoclasts mediated by TGF-β. Nat Med 1996; 7:1132-1136.
6. Pfeilschifter J, Wolf O, Naumann A, Minne HW, Mundy GR. Chemotactic response of osteoblast-like cells to transforming growth factor β. J Bone Miner Res 1990; 5:825-830.
7. Mundy GR, Rodan SB, Majeska RJ, De Martino S, Trimmier C, Martin TJ, Rodan GA. Unidirectional migration of osteosarcoma cells with osteoblast characteristics in response to products of bone resorption. Calcif Tissue Int 1982; 34:542-546.
8. Mundy GR, Poser JW. Chemotactic activity of the gamma-carboxyglutamic acid containing protein in bone. Calcif Tissue Int 1985; 35:164-168.
9. Canalis E, Pash J, Varghese S. Skeletal growth factors. Crit Rev Eukaryot Gene Expr 1993; 3:155-166.
10. Canalis E, McCarthy T, Centrella M. Growth factors and the regulation of bone remodeling. J Clin Invest

1989; 81:277-281.
11. Eriksen EF, Axelrod DW, Melsen F. Bone histology and bone histomorphometry. In: Eriksen EF, Axelrod DW, Melsen F (eds) Bone Histomorphometry. Raven Press, New York, NY, USA; 1994:13-20.
12. Jee WSS. The skeletal tissues. In: Weiss L (ed) Histology, Cell and Tissue Biology. Elsevier Biomedical, New York, NY, USA; 1983: 220-255.
13. Burr DB, Martin RB, Schaffler MB, Radin EL. Bone remodeling in response to *in vivo* fatigue microdamage. J Biomech 1985; 18:189-200.
14. Burr DB, Martin RB. Calculating the probability that microcracks initiate resorption spaces. J Biomech 1993; 18:189-200.
15. Noble B. Bone microdamage and cell apoptosis. Europ Cells Mat 2003; 6:46-56.
16. Burr DB. Targeted and nontargeted remodeling. Bone 2002; 30:2-4.
17. Parfitt AM. Skeletal heterogeneity and the purposes of bone remodeling: implications for the understanding of osteoporosis. In: Marcus R, Feldman D, Kelsey J (eds) Osteoporosis. Academic, San Diego, CA, USA; 1996:315-329.
18. Nijweide PJ, Burger EH, Klein-Nulend J. The osteocyte. In: Bilezikian JP Raisz LG, Rodan GA (eds) Principles of Bone Biology, 2nd ed. Academic Press, San Diego, CA, USA; 2002:93-107.
19. van der Plas A, Aarden EM, Feyen JH, de Boer AH, Wiltink A, Alblas MJ, de Leij L, Nijweide PJ. Characteristics and properties of osteocytes in culture. J Bone Miner Res 1994; 9:1697-1704.
20. Bonewald LF. Establishment and characterization of an osteocyte-like cell line, MLO-Y4. J Bone Miner Metab 1999; 17:61-65.
21. Marotti G. The structure of bone tissues and the cellular control of their source. Ital J Anat Embryol 1996; 101:25-79.
22. Civitelli R, Beyer EC, Warlow PM, Robertson AJ, Geist ST, Steinberg TH. Connexin43 mediates direct intercellular communication in human osteoblastic cell networks. J Clin Invest 1993; 91:1888-1896.
23. Rubin CT, Lanyon LE. Osteoregulatory nature of mechanical stimuli: function as determinant for adaptive bone remodeling. J Orthop Res 1987; 5:300-310.
24. Jorgensen NR, Teilmann SC, Henriksen Z, Civitelli R, Sorensen OH, Steinberg TH. Activation of L-type calcium channels is required for gap junction-mediated intercellular calcium signaling in osteoblastic cells. J Biol Chem 2003; 278:4082-4086.
25. Jiang JX, Cheng B. Mechanical stimulation of gap junctions in bone osteocytes is mediated by prostaglandin E2. Cell Commun Adhes 2001; 8:283-288.
26. Ziros PG, Gil AP, Georgakopoulos T, Habeos I, Kletsas D, Basdra EK, Papavassiliou AG. The bone-specific transcriptional regulator Cbfa1 is a target of mechanical signals in osteoblastic cells. J Biol Chem 2002; 277:23934-23941.
27. Turner CH, Robling AG, Duncan RL, Burr DB. Do bone cells behave like a neuronal network? Calcif Tissue Int 2002; 70:435-442.
28. Pavalko FM, Norvell SM, Burr DB, Turner CH, Duncan RL, Bidwell JP. A model for mechanotransduction in bone cells: the load bearing mechanosomes. J Cell Biochem 2003; 88:104-112.
29. Smit TH, Burger EH. Is BMU-coupling a strain regulated phenomenon? A finite element analysis. J Bone Miner Res 2000; 15:301-307.
30. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman, AB Eriksen EF, Ish-Shalom S, Genant HK Wang O, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 2001; 344:1434-1441.
31. Orwoll ES, Scheele WH, Paul S, Adami S, Syversen U, Diez-Perez A, Kaufman JM, Clancy AD, Gaich GA. The effect of teriparatide [human parathyroid hormone (1-34)] therapy on bone density in men with osteoporosis. J Bone Miner Res 2003; 18:9-17.
32. Bradbeer JN, Arlot ME, Meunier PJ, Reeve J. Treatment of osteoporosis with parathyroid peptide (hPTH 1-34) and oestrogen: increase in volumetric density of iliac cancellous bone may depend on reduced trabecular spacing as well as increased thickness of packets of newly formed bone. Clin Endocrinol (Oxf) 1992; 37:282-289.
33. Jiang Y, Zhao JJ, Mitlak BH, Wang O, Genant HK, Eriksen EF. Recombinant human parathyroid hormone (1-34) [teriparatide] improves both cortical and cancellous bone structure. J Bone Miner Res 2003; 18:1932-1941.
34. Dempster DW, Cosman F, Kurland ES, Zhou H, Nieves J, Woelfert L, Shane E, Plavetic K, Muller R, Bilezikian J, Lindsay R. Effects of daily treatment with parathyroid hormone on bone microarchitecture and turnover in patients with osteoporosis: a paired biopsy study. J Bone Miner Res 2001; 16:1846-1853.
35. Oxlund H, Ejersted C, Andreassen TT, Torring O, Nilsson MH. Parathyroid hormone (1-34) and (1-84) stimulate cortical bone formation both from periosteum and endosteum. Calcif Tissue Int 1993; 53:394-399.
36. Zanchetta JR, Bogado CE, Ferretti JL, Wang O, Wilson MG, Sato M, Gaich GA, Dalsky GP, Myers SL. Effects of teriparatide [recombinant human parathyroid hormone (1-34)] on cortical bone in postmenopausal women with osteoporosis. J Bone Miner Res 2003; 18:539-543.
37. Uusi-Rasi K, Semanick LM, Zanchetta JR, Bogado CE, Eriksen EF, Sato M, Beck TJ. Effects of teriparatide [rhPTH (1-34)] treatment on structural geometry of the proximal femur in elderly osteoporotic women. Bone 2005; 36:948-958.
38. Lane NE, Sanchez S, Modin GW, Genant HK, Pierini E, Arnaud CD. Bone mass continues to increase at the hip after parathyroid hormone treatment is discontin-

ued in glucocorticoid-induced osteoporosis: results of a randomized controlled clinical trial. J Bone Miner Res 2000; 15:944-951.
39. Hodsman AB, Hanley DA, Ettinger MP, Bolognese MA, Fox J, Metcalfe AJ, Lindsay R. Efficacy and safety of human parathyroid hormone-(1-84) in increasing bone mineral density in postmenopausal osteoporosis. J Clin Endocrinol Metab 2003; 88:5212-5220.
40. Hodsman AB, Fraher LJ, Ostbye T, Adachi JD, Steer BM. An evaluation of several biochemical markers for bone formation and resorption in a protocol utilizing cyclical parathyroid hormone and calcitonin therapy for osteoporosis. J Clin Invest 1993; 91:1138-1148.
41. Hodsman AB, Steer BM. Early histomorphometric changes in response to parathyroid hormone therapy in osteoporosis: evidence for de novo bone formation on quiescent cancellous surfaces. Bone 1993; 14:523-527.
42. Divieti P, Inomata N, Chapin K, Singh R, Juppner H, Bringhurst FR. Receptors for the carboxyl-terminal region of PTH(1-84) are highly expressed in osteocytic cells. Endocrinology 2001; 142:916-925.
43. Dobnig H, Turner RT. Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. Endocrinology 1995; 136:3632-3638.
44. Leaffer D, Sweeney M, Kellerman LA, Avnur Z, Krstenansky JL, Vickery BH, Caulfield JP. Modulation of osteogenic cell ultrastructure by RS-23581, an analog of human parathyroid hormone (PTH)-related peptide-(1-34), and bovine PTH-(1-34). Endocrinology 1995; 136:3624-3631.
45. Keller HJ, Kneissel M. 2SOST is a target gene for PTH in bone. Bone 2005; 37:148-158.
46. Poole KES, van Bezooijen RL, Loveridge M, Hamersma H, Papapoulos S, Löwik CW, Reeve J. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J 2005; 19:1842-1844.
47. Jones DB, Broeckmann E, Pohl T, Smith EL. Development of a mechanical testing and loading system for trabecular bone studies for long term culture. Europ Cells Mat 2003; 5:48-60.
48. de Boer J, van Blitterswijk C, Lowik C. Bioluminescent imaging: emerging technology for non-invasive imaging of bone tissue engineering. Biomaterials 2006; 9:1851-1858.