The mechanism of transduction of mechanical strains into biological signals at the bone cellular level

G. Marotti, C. Palumbo
Department of Anatomy and Histology, University of Modena and Reggio Emilia, Italy

As appears from the literature, the majority of bone researchers consider osteoblasts and osteoclasts the only very important bony cells. In the present report we provide evidence, based on personal morphofunctional investigations, that such a view is incorrect and misleading. Indeed osteoblasts and osteoclasts undoubtedly are the only bone forming and bone reabsorbing cells, but they are transient cells, thus they cannot be the first to be involved in sensing both mechanical and non-mechanical agents which control bone modeling and remodeling processes. Briefly, according to our view, osteoblasts and osteoclasts represent the *arms of a worker*; the actual *operation center* is constituted by the cells of the osteogenic lineage in the resting state. Such a resting phase is characterized by osteocytes, bone lining cells and stromal cells, all connected in a functional syncytium by gap junctions, which extends from the bone to the vessels. We named this syncytium the *Bone Basic Cellular System* (BBCS), because it represents the only permanent cellular background capable first of sensing mechanical strains and biochemical factors and then of triggering and driving both processes of bone formation and bone resorption. As shown by our studies, signalling throughout BBCS can occur by *volume transmission* (VT) and/or *wiring transmission* (WT). VT corresponds to the routes followed by soluble substances (hormones, cytokines etc.), whereas WT represents the diffusion of ionic currents along cytoplasmic processes in a neuron-like manner. It is likely that non-mechanical agents first affect stromal cells and diffuse by VT to reach the other cells of BBCS, whereas mechanical agents are first sensed by osteocytes and then issued throughout BBCS by WT.

Key words: osteogenic cells, osteoclasts, cytokines, mechanical strains.

Correspondence: Gastone Marotti,
Dipartimento di Anatomia e Istologia Policlinico,
Largo del Pozzo, 71 41100 Modena, Italy
Tel: +39.059.4224800.
Fax: +39.059.4224861.
E-mail: gmarotti@unimore.it

European Journal of Histochemistry
2007; vol. 51 supplement 1:15-20

It is a well established fact that, under the control of mechanical agents (body weight, force of gravity, muscular tone and strength) and non-mechanical agents (hormones, vitamins, cytokines, growth factors), bone cells regulate bone homeostasis and take part in the maintenance of mineral homeostasis, by means of three processes: bone growth, bone modeling and bone remodeling. Bone growth and bone modeling are only devoted to the regulation of bone homeostasis, whereas bone remodeling takes part in the regulation of bone homeostasis as well as of mineral homeostasis, by respectively improving bone structure in response to mechanical demands and setting free calcium and phosphate ions during the reabsorbing phase.

Frost's mechanostat theory (Frost, 1987) and Utah paradigm (1985) have greatly rationalized bone modeling and remodeling processes and what they involve at the bone macroscopic level. However what happens at the cellular level still remains to be defined. We do not know, for instance: a) how mechanical agents and non-mechanical agents interact at the cellular level; b) which is the mechanism of transduction of mechanical strains into biological signals; updated literature ascribes to osteocytes the function of sensing the strains induced into the bone matrix by mechanical stresses but, as we will discuss below, all cells of the osteogenic system are likely to be affected by mechanical strains; c) how osteocytes transmit mechanical stimuli to, and interact with, the other bone cells.

In the attempt to answer these questions we will first summarize the results of the morphofunctional investigations we carried out on the cells of the osteogenic lineage during the last three decades. Then we will discuss some functional implications.

The cells of the osteogenic lineage: morphological aspect and function

In the early 1970s, we showed that the exponential decrement of the appositional growth rate,

which has been shown to occur during osteon formation by means of triple fluorochrome technique (Manson and Waters, 1963; Marotti and Camosso, 1968), depends on the diminution in size of the osteoblasts and their progressive flattening. At the beginning of osteon formation, when the appositional rate is high, the osteoblasts are big and prismatic, whereas towards the end of osteon formation, when the rate is low, they are smaller and flat (Marotti, 1976).

Since these facts were also observed in trabecular bone, our conclusion was that the rate at which the bone tissue is laid down depends on the ratio between the *volume* of the osteoblasts and their secretory territory: the greater the osteoblast volume and the smaller its *secretory territory*, the higher the rate of bone apposition (Marotti, 1976).

Additionally we showed that, during the edification of osteons, also the osteocytes decrease in size, in parallel to the decrement of osteoblast dimension and the appositional growth rate. This finding implies that the size of the osteocytes strictly depends on the size of the osteoblasts from which they differentiate: the bigger the osteoblasts the larger the size of the osteocytes (Marotti, 1976). The functional meaning of this fact as yet to be established. However we found, in human osteons, that the decrement in size of osteocytes from the cement line towards the Haversian canal is paralleled by a thinning of *osteocytic-loose* (collagen poor) lamellae and, consequently, by a diminution of the distance between *non-osteocytic-dense* (collagen rich) *lamellae,* whose thickness does not significantly change throughout the osteonic wall. Mechanically speaking, this fact involves an increase in collagen fibers, namely in bone strength, along the bone surfaces where stresses and strains reach the highest values (Ardizzoni *et al.,* 1999).

In more recent years, we showed by transmission and scanning electron microscopes that the arborization of osteocytes is asymmetrical as regards both number and length of cytoplasmic processes. Vascular dendrites (those radiating toward the bone vascular surface) are more numerous (Marotti *et al.,* 1985) and incomparably longer than mineral dendrites (those radiating towards the opposite surface) (Palumbo, 1986; Palumbo *et al.,* 1990a, 1990b). Therefore osteocyte appear to be polarized cells, towards the bone surface where they come into contact whether osteoblasts or bone lining cells, according to which the bone surface is growing or resting.

Additionally we found that the number of osteocyte vascular dendrites coming into contact with each osteoblast is inversely proportional to the osteoblast size, namely to its bone forming activity. This fact suggests a possible inhibitory effect of osteocytes on osteoblasts (Marotti *et al.,* 1992).

In subsequent series of transmission electron microscope investigations we found that also bone-associated stromal cells are dendritic elements. They form a continuous cytoplasmic network which extends from endothelial cells to bone lining cells or osteoblasts (Palazzini *et al.,* 1998). Since gap junctions (actually considered as electrical synapses, when active) were observed throughout all cells of the osteogenic system, including stromal cells, it seems likely that not only osteocytes but all cells of the osteogenic lineage are functionally connected in a syncytium.

On the basis of these findings, we postulated that the transmission of signals throughout the cells of the osteogenic system may occur by means of two mechanisms: *volume transmission* (VT) and *wiring transmission* (WT). VT corresponds to the well-known routes followed by hormones, cytokines and growth factors to reach the bone cells. The novelty of our hypothesis lies in the suggestion that the cells of the osteogenic lineage may communicate reciprocally and modulate their activity by WT, namely in a neuron-like manner (Marotti *et al.* 1993, 1996; Marotti, 1996). Indeed some similarities do exist between osteocytes and neurons. Mineral cytoplasmic processes of osteocytes resemble neuronal dendrites in that they are shorter, thicker and may contain cell organelles, whereas osteocyte vascular cytoplasmic processes are longer, slender and do not contain organelles, thus resembling neuronal axons. Transmission of signals through osteocytes seems to occur by gap junctions instead of synapses, though it has been shown that osteocytes produce typical neurotransmitters like nitric oxide (Zaman *et al.,* 1999) and amino acid glutamate (Skerry, 1999).

In recent years we provide evidence that WT really occurs along osteocytes in amphibian (Rubinacci *et al.,* 1998) as well as in murine (Rubinacci *et al.,* 2002) cortical bone. Metatarsal bones, placed in an experimental chamber in *ex vivo* conditions, were subjected by a mechanical stimulator to pulsing axial loading by varying the loading parameters: amplitude and frequency. A 200 micra hole was

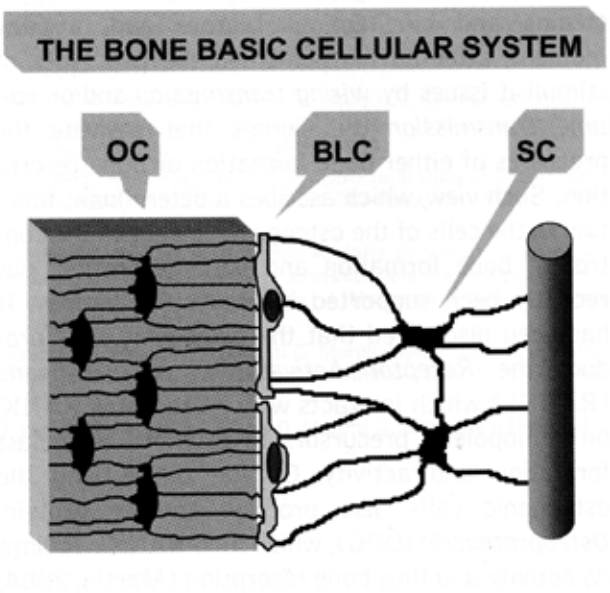

Figure 1. Schematic drawing of the cells of the osteogenic lineage in the resting phase, the so called *Bone Basic Cellular System*. From left to right: osteocytes (OC), bone lining cells (BLC), stromal cells (SC) and a vascular capillary. This network of cells forms a functional syncytium since they are all joined by *gap junctions*. It is suggested that this syncytium is capable of sensing both mechanical strains and biochemical factors and, at any moment, after having combined the two types of stimuli, it issues by *wiring* and/or *volume transmission* the appropriate signals that activate bone formation or bone resorption.

previously drilled through the metatarsal cortex and the ionic currents entering the hole were monitored by a two-dimensional vibrating probe system.

The following results were obtained. *Before loading*: signal of 15.5±4.6 micronA/cm^2 was recorded for living bone; no signal was detected for dead bone (i.e. dead osteocytes). *After loading under 5 g at 1Herz*: a) dead bone, too, exhibited an ionic current, but living bone drove a current about 4 times higher; b) the time pattern decay in dead bone tended linearly to 0 within 70'; in living bone it decreased exponentially, approaching the basal values within 15' and afterwards it remained steady over time. By increasing the load from 0.7 to 12 g at a fixed frequency of 1Hz, the current increased with increasing loads up to 8 g only, but under higher loads it persisted at a higher level over time. By increasing the frequencies from static to 2Hz at a fixed load of 5 g, we recorded the same results obtained by increasing the loads at a constant frequency. Static load did not induce any current. Briefly, these findings indicate that: 1) bone strains induce an ionic streaming potential within the osteocyte lacuno-canalicular system that activates osteocytes which, in turn, increase and maintain steady the basal current; 2) osteocytes are capable of summarizing the whole amount of energy they receive. The fact that osteocyte effect persists over time suggests the hypothesis that, under physiological loads, they have an inhibitory activity on the other cells of the osteogenic lineage and, consequently, on bone remodeling.

Discussion and functional implications

It resulted from our morphological investigations that the osteogenic cellular system (stromal cells, osteoblasts or bone lining cells, osteocytes) constitutes a functional syncytium whose variously shaped cells play different roles and have different relationships with the surrounding environment. The cytoplasmic network of stellate stromal cells is immersed in the interstitial fluid, and extends from vascular endothelium to the cells carpeting the bone surface, i.e. osteoblasts or bone lining cells. Osteocytes display an asymmetrical dendrite arborization polarized towards osteoblasts or bone lining cells, and are enclosed inside bone microcavities filled with the bone fluid compartment, having a different composition from the perivascular interstitial fluid where stromal cells are located. Osteoblasts and bone lining cells form cellular laminae in between two networks of dendrites: on their vascular side they are in contact with stromal cell processes, whereas on their bony side they are in contact with osteocyte vascular dendrites. Moreover osteoblasts and bone lining cells separate the bone fluid compartment from the perivascular interstitial fluid.

In our opinion, one of the biggest mistake made by the majority of researchers, particularly molecular biologists, was to consider the bones only in the active phases of formation and/or resorption, and thus only osteoblasts and osteoclasts were deeply studied. We should, however, bear in mind that osteoblasts and osteoclasts are transient cells; they constitute the arms *of a worker*. If we wish to detect where is the *operation center*, in order to understand how the processes of bone formation and bone resorption are first triggered and then modulated, we must focus our investigations on the events occurring in the bone cellular system starting from the resting, steady state.

According to our morphological studies, the resting phase is characterized by osteocytes, bone lining cells, and stromal cells, all connected in a functional syncytium, which extends from the bone to the

endothelial lining (Figure 1). We named this syncytium the *Bone Basic Cellular System* (BBCS) because it represents the cellular background capable of triggering and driving both processes of bone formation and bone resorption, under the control of mechanical and non-mechanical agents. It is likely that mechanical agents are first sensed by osteocytes and, in second instance, probably also by the other cells of the osteogenic lineage, whereas non-mechanical agents first affect stromal cells and then diffuse into the bone fluid volume to reach the bone lining cells and finally the osteocytes via their canalicular system. In our view BBCS represents the *bone operation center*. This view is supported by the following facts: a) bone overloading and unloading respectively induce modeling-dependent bone gain and remodeling-dependent bone loss also in adult skeleton, in which no or few osteoblasts and osteoclasts are present whereas BBCS is surely present, thus suggesting it intervenes in activating both bone formation and bone resorption; b) bone resorption was found to occur in regions less subjected to mechanical loading in biochemical osteoporoses (Lozupone and Favia, 1988; Bagi and Miller, 1994), whereas in disuse osteoporosis it takes place uniformly throughout the skeletal segments (Lozupone and Favia, 1982; Bagi and Miller, 1994), thus indicating that osteoclast activity is activated and driven by local signals which can but be issued by BBCS.

As regards osteoclasts, they are free cells that never become part of the osteogenic cell network; on the contrary, it seems likely that they should destroy stromal cells and bone lining cells, before reabsorbing the bone matrix and osteocytes. Therefore, strictly speaking, osteoclasts do not pertain to bone cells. They instead appear to be workers specialized in bone destruction and, when their activity is needed, BBCS calls them, probably by secreting osteoclast activating cytokines (RANKL), and tell them where, when and how long they have to work (Palumbo *et al.*, 2001). Osteoclasts are also under the control of blood derived systemic factors, whereas they should not be capable of sensing mechanical strains being free cells.

In conclusion, according to our view all processes of bone formation and bone resorption, occurring in response to mechanical agents and non-mechanical agents, are triggered, modulated, and stopped by the BBCS. This appears to be the real *bone operations center* capable of sensing both mechanical strains and biochemical factors and, at any moment, after having combined the two types of stimuli it issues by *wiring transmission* and/or *volume transmission* the signals that activate the processes of either bone formation or bone resorption. Such view, which ascribes a determinant function to the cells of the osteogenic lineage in the control of bone formation and bone resorption, has recently been supported by molecular biology. It has been discovered that the osteogenic cells produce the *Receptor Activator of NF-kB ligand* (RANKL) which interacts with its receptor, RANK, on hemopoietic precursors to promote osteoclast formation and activity. On the other hand the osteogenic cells also produce another protein, *Osteoprotegerin* (OPG), which bind RANKL to limit its activity and thus bone resorption (Martin, 2004; Hofbauer *et al.*, 2004).

References

Ardizzoni A, Muglia MA, Marotti G. Osteocyte size-lamellar thickness relationships. 8th Congress of ISBM, J Bone Miner Res 1999.

Bagi CM, Miller SC. Comparison of osteopenic changes in cancellous bone induced by ovariectomy and/or immobilization in adult rats. Anat Rec 1994; 239:243-54.

Frost H.M. Bone "mass" and the "mechanostat": A proposal. Anat Rec 1987; 219:1-9.

Frost HM. Introduction to a new skeletal physiology. Vols I, II. Pajaro Group, Pueblo, CO, 1995.

Hofbauer LC, Kuhne CA, Viereck V. The OPG/RANKL/RANK system in metabolic bone diseases. J Musculoskel Neuron Interact 2004; 4:268-75.

Lozupone E, Favia A. Density of trabecular framework and osteogenic activity in the spongiosa of long bones subjected to drastic changes in mechanical loading. Anat Anz 1982; 152:245-61.

Lozupone E, Favia A. Distribution of resorption processes in the compacta and spongiosa of bones from lactating rats fed a low-calcium diet. Bone 1988; 9:215-24.

Manson JD, Waters NE. Maturation rate of osteon of the cat. Nature, Lond 1963; 200:89-490.

Marotti G. Decrement in volume of osteoblasts during osteon formation and its effect on the size of the corresponding osteocytes. In: "Bone histomorphometry" Meunier P.J ed.; Armour Montagu, Levallois, 1976, pp. 385-97.

Marotti G. The structure of bone tissues and the cellular control of their deposition. Italian J Anat Embryol 1996; 101:25-79.

Marotti G, Camosso ME. Quantitative analysis of osteonic bone dynamics in the various periods of life. In "Les Tissus Calcifiés", eds. Milhaud G, Owen M, Blackwood HJJ, SEDES, Paris, 1968, pp. 423-7.

Marotti G, Ferretti M, Muglia MA, Palumbo C, Palazzini S. A quantitative evaluation of osteoblast-osteocyte relationships on growing endosteal surface of rabbit tibiae. Bone 1992; 13:363-8.

Marotti G, Palazzini S, Palumbo C. Evidence of a twofold regulation of osteoblast activity: "Volume transmission" and "Wiring transmission". Calcif Tissue Int 1993; 53:440.

Marotti G, Palazzini S, Palumbo C, Ferretti M. Ultrastructural evidence of the existence of a dendritic network throughout the cells of the osteogenic lineage: the novel concept of wiring- and volume-transmission in bone. Bone 1996; 19(Suppl. 3):151S.

Marotti G, Remaggi F, Zaffe D. Quantitative investigation on osteocyte

canaliculi in human compact and spongy bone. Bone 1985; 6:335-7.

Martin TJ. Parine regulation of osteoclast formation and activity: Milestones in discovery. J Musculoskel Neuron Interact 2004; 4:243-53.

Palazzini S, Palumbo C, Ferretti M. Marotti G. Stromal cell structure and relationships in perimedullary spaces of chick embryo shaft bones. Anat Embryol 1998; 197:349-57.

Palumbo C. A three-dimensional ultrastructural study of osteoid-osteocytes in the tibia of chick embryos. Cell Tissue Res 1986; 246:125-31.

Palumbo C, Ferretti M, Ardizzoni A, Zaffe D, Marotti G. Osteocyte-osteoclast morphological relationships and the putative role of osteocytes in bone remodeling. J Musculoskel Neuron Interact 2001; 1:327-32.

Palumbo C, Palazzini S, Marotti G. Morphological study of intercellular junctions during osteocyte differentiation. Bone 11; 1990a:401-6.

Palumbo C, Palazzini S, Zaffe D, Marotti G. Osteocyte differentiation in the tibia of newborn rabbit: an ultrastructural study of the formation of cytoplasmic processes. Acta Anat 1990b; 137:350-8.

Rubinacci A, Covini M, Bisogni C, Villa I, Galli M, Palumbo C, et al. Bone as an ion exchange system: evidence for a limk between mechanotransduction and metabolic needs. Am J Physiol Endocrinl Metab 2002; 282:E851-64.

Rubinacci A, Villa I, Dondi Benelli F, Borgo E, Ferretti M, Palumbo C, et al. Osteocyte-bone lining cell system at the origin of steady ionic current in amphibian bone. Calc Tissue Int 1998; 63:331-9.

Skerry TM. Signalling pathways activated during functional adaptation of the skeleton to mechanical loading suggest a role for excitatory amino acid glutamate. 1st International Workshop on Musculoskeletal Interactions, Santorini Greece, I.S.M.N.I., 1999, p. 20.

Zaman G, Pitsillides AA, Rawlinson SCF, Suswillo RFL, Mosley JR, Cheng MZ, et al. Mechanical strain stimulates nitric oxide production by rapid activation of endothelial nitric oxide synthase in osteocytes J Bone Miner Res 1999; 14:1123-31.