Osteocytes: On the central role of these cells in osseous pathobiology

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Abstract

The main targets for the comprehension of bone pathobiology were focused in osteoblasts and clasts, but in recent years it has shifted to the osteocytes — as mechanotransductors of the bone tissue, from the three-dimensional network, by interconnecting its extensions linking a cell to other 20 to 40, like a neural network. By mechanotransduction and from mediators as sclerostin and RANKL, the osteocytes may influence bone pathobiology by interfering with the activity of osteoblasts and clasts. When more bone is necessary, osteocytes release less sclerostin, when it is necessary to inhibit bone formation, osteocytes release more sclerostin. RANKL is connected to local osteoclastogenesis in order to have more cells capable of reabsorbing the mineralized matrix. New therapeutic ways of controlling the metabolic bone diseases have been targeted at these mediators. Studying the presence and the specific effects of sclerostin and RANKL in osseointegration can lead to greater detailing of their biological phenomena.

Keywords: Osteocytes. Mechanotransduction. Bone biology. Sclerostin. RANKL.

How to mention this article: Consolaro A. Osteocytes: On the central role of these cells in osseous pathobiology. Dental Press Implantol. 2012 Apr-June;6(2):20-8.

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Submitted on: 03/20/2012
Reviewed and received on: 03/28/2012

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Introduction

In bone biology and comprehension of associated diseases, osteocytes for long were rejected to a secondary role, because it was believed that they were included in the mineralized bone matrix and did not participate in bone metabolism and in responses to stimuli and aggression.

As shown in numerous studies from the last five years, there is a strong influence of osteocytes in bone remodeling and, for this reason, they must participate in the mechanism of initial osseointegration and its maintenance over the years of dental implants use.

The dendritic shape of the osteocyte makes it comparable to a neuron, putting it in contact with up to 40 to 50 cells simultaneously, generating a three dimensional network among themselves communicating very efficiently. In any deformation the bone may suffer from deflections resulting from compression and tractions, the osteocytes of this communicative network will act as mechanotransductors by excellence. Osteocytes also participate in the control of bone metabolism by releasing mediators that reach the bone surfaces, strongly influencing the activity of osteoblasts and clasts in trabecular and cortical areas.

The osteocytes are derived from osteoblasts

Osteoblasts, which originate osteocytes, are cells with mesenchymal origin that differentiate by stimulation of mediators still in the embryo or fetus. The main mediator of differentiation and synthesizing activity in this intrauterine phase are the BMPs, or osteomorphogenetic proteins. The mediators that determine the shape of organs and structures in the embryonic stage can also be identified as morphogens, such as bone morphogenetic proteins.

In an environment of osseodifferenciation and synthesis of bone matrix, most of the molecules of stimulating mediators of these phenomena are eventually included in the extracellular bone matrix to be mineralized later. Thus, one can say that any mineralized bone matrix has naturally bone morphogenetic proteins in its composition, in a greater or lesser amount as part of its natural composition.

Once the skeleton is formed and adulthood is established, osteoblasts and osteocytes remain present in the bone environment. In bone surfaces many osteoprogenitor cells remain along with pre-osteoblasts and tissue stem cell, formerly named undifferentiated mesenchymal cells. In the bone marrow, contained and protected by cortical and trabecular there are many tissue stem cells, which give rise to new bone cells almost infinitely.

Osteoblasts on the surfaces of trabecular and cortical bone are polyhedral cells arranged next to each other like a real fence, railing or lattice. Its polyhedral shape, with several facets or sides, allows the production of bone matrix in one of its sides; in another one, receptors are exposed to mediators located in adjacent connective tissue or marrow tissue. At the same time, osteoblasts laterally contact themselves and interact with other osteoblasts to form a real layer of cells coating on bone surfaces.

In certain conditions, osteoblasts synthesize and mineralize the bone matrix; in other ones, as in inflamed and stressed areas, mediators can induce osteoblasts to move from the bone surface. Before leaving the surface, osteoblasts release enzymes such as collagenase, to remove the last bone layer deposited by them and still not mineralized. Although osteoblasts move from the surface, they remain close and command the activity of clasts within an osteoremodeling unit, or BMU.

In this bone matrix deposition many osteoblasts eventually end up included in gaps called osteoplasts (Fig 1, 2 and 3). It was believed for many years that these cells would be trapped, almost by a passive mechanism,

as if they had lost the moment to depart, and got involved in the newly deposited matrix. The passive role of osteocytes was proved untrue. On the contrary, these cells seem to perform a central role in controlling bone remodeling and opposite reactions to certain stimuli.

Osteocytes: morphology and functions

Osteocytes represent from 90 to 95% of the bone cells in an adult. 15 These cells are included in the mineralized bone matrix (Fig 1, 2 and 3) and now, as with osteoblasts and clasts, we also have greater knowledge about the osteocytes and their functions.

The osteocytes are regularly distributed within bone matrix gaps, also known as osteoplasts, and communicate with the cells of the bone surface through extensions in the tubules at 100 to 300 nm thickness.[3,4,5] They form a web with their extensions, a network comparable to the neural network in the central neural system (Fig 1, 2, 3).

Within these tubules, where the cytoplasmic processes of each cell are (Fig 1, 2 and 3), circulates a fluid tissue that carries nutrients and mediators. These canaliculi with its working fluid and its extensions communicate the osteocytes with each other and interconnected with the surface cells of cortical and trabecular bone, in addition to resident cells of the bone marrow.[10] This communication can be cell-cell by means of specialized junctions or mediators (Figs 1, 2 and 3).

The concept of mechanotransduction

Cells have a cytoskeleton, one scaffold responsible for maintaining normal cell shape, movement and migration. The cytoskeleton is composed of well-structured proteins, divided into three main groups, according to their molecular weight and spatial structure: microtubules, microfilaments and intermediate filaments.

In any system, the balance provided by the annulation of all its intrinsic strength results in a force equal to zero and it is called tensegrity. The shape of a cell tends to be the same, as a result of the balance of internal and external forces. To this state of equilibrium or stability is given the name cellular tensegrity.

When tensegrity is lost by compression, the cytoskeleton, like any other natural system, tends to return to its previous state, spurring a series of events for this purpose. The release of chemical mediators to induce cellular and tissue phenomena in itself or around it, is part of the process by which cells tend to restore their tensegrity. This determines stable shape, the morphology pattern of an object or system, especially of a cell.

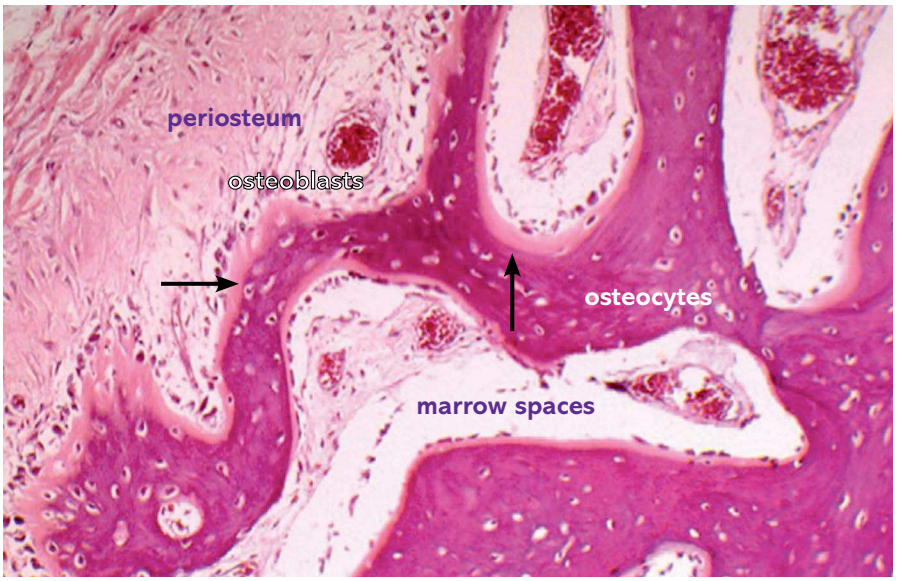

Figure 1 - The osteocyte network participates of the cellular functional control on bone surface, such as the clast and osteoblasts. The cytoplasmatic prolongations arrive at the canaliculi and make contact with the surface cells or act via mediators (HE; 40X).

The breaking of tensegrity modifies the permeability of the cell membrane and results in the activation of intracellular metabolic pathways, releasing substances that act as mediators that induce phenomena of cellular, tissue and / or vascular nature. These substances are cytokines, growth factors and arachidonic acid products. By this mechanism, a physical event, such as forces, turns into biochemical and biological events: this transformation is known as mechanotransduction.

Bone Mechanotransductors: Osteocytes

The osteocytes network form a very sensitive 3D system that uptakes bone deformities. Any change in bone form during skeleton function can be captured by this sensitive network or web of osteocytes, and extensions or mechanotransduction detection system. Exercise can increase bone structure by mechanical stimuli, initially, on this network scavenging strain.

The osteocytes individually pick up signals by mechanical deformation of their cytoskeleton. At the same time, the network in which each osteocyte participates, distributed throughout the bone structure, picks up deformations, overloads, deflections and limitations of nutrients. The deformation of the cytoskeleton, the restriction of oxygen and of nutrient stress the osteocytes, which release mediators to communicate with other osteoblasts and clasts on the bone surface and induce them to reactive or adaptive phenomena.

When we deform, compress or strain the bone as happens during orthodontic movement, we put the osteocytes in mechanical stress and, thus, it increases the production of secreted and circulating mediators through the fluid that circulates in the canaliculi (Fig 1, 2 and 3) and from there to the respective periodontal and bone surfaces. Although included in the mineralized bone matrix in their osteoplasts, the osteocytes and its communicating network — by direct contact or mediators — can stimulate or inhibit bone formation and bone resorption in the "distant" cortical bone surface (Fig 3). The osteocytes in the bone marrow inside the bone, can influence the higher or lower production of clastic cells, or osteoclastogenesis.

The osteocytes therefore have strong influence in function of bone adapting its shape as functional demand determination, transforming the mechanical stimuli into biochemical events, a phenomenon known as mechanotransduction.[13] The osteocytes have also function in regulating mineral metabolism[9] and also induce changes in the properties of the bone matrix around it[12] — but these functions were already better known.

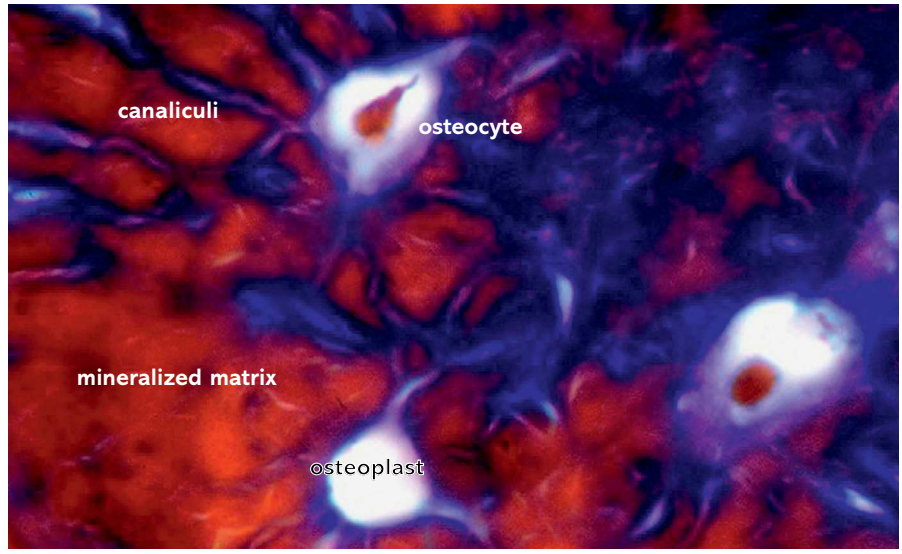

Figure 2 - The osteocytes have many cytoplasmatic prolongations, which intercommunicate with the mineralized matrix with other 20 to 40-50 cells and they detect minimal structural deformations and act as mechanotransductors. They occupy lacunnae known as osteoplasts and the prolongations spread out as canaliculi, where mediators circulate in a tissue fluid, which performs ionic Exchange with the mineralized extracellular matrix. (Mallory; 100X).

The skeleton is able to continuously adapt to mechanical loads by the addition of new bone to increase the ability to resist or remove bone in response to a lighter load or lack of use.[6,8] The osteocytes have a high interconnectivity and are considered the bone mechanotransductors.

Osteocytes increases glucose-6-dehydrogenase phosphatase after a few minutes of load,[18] a marker for increased metabolism, as it occurs in cells associated with bone surface. Seconds after the applied load on the osteocytes, nitric oxide prostaglandins and other molecules such as ATP[1] are increased.

Therefore, osteocytes, when facing induced loads, have the ability to release mediators, which stimulate the precursors of clasts or osteoclastogenesis to differentiate into new clasts increasing the rate of resorption. Among these mediators the M-CSF or stimulating factor of colonies for macrophages and RANKL should be highlighted.[14] It can be argued that osteocytes can command the activities of the clasts on bone surfaces according to functional demand. The set or lacunocanalicular osteocyte system can be seen as a real endocrine body.[4]

Osteocytes, implants and osseointegration

In micro-bone lesions that occur daily, osteocytes die by apoptosis or necrosis. Osteocytes die for having finished their natural life cycle stimuli or by external agents such as heat or dryness in the bone tissue during surgical procedures. Apoptosis is a cell death controlled by specific genes of the cell itself, while necrosis is cell death by external agents, tearing it, crumpling it or taking their nutrition by breaking vases. Some environmental factors and mediators can induce cell apoptosis.

The death of osteocytes in areas with 1-2 mm damage, such as microfractures, can generate mediators that stimulate clasts, especially RANKL,[7] a group TNF cytokine. Preserving the osteocytes is to prevent bone reabsorption and clinicians should know this information to take better care of the surgical margins in bone surfaces. In orthodontics many procedures are surgical.

In Implantology, the preparation of stores for receiving the dental implants must follow protocols for maximum preservation of the viability of biological bone cells in cutting areas. Preserving the osteocytes in bone surgical margins involves avoiding any need for prior resorption of mineralized matrix before starting the osseointegration. Whenever the osteocytes are dead in their gaps,

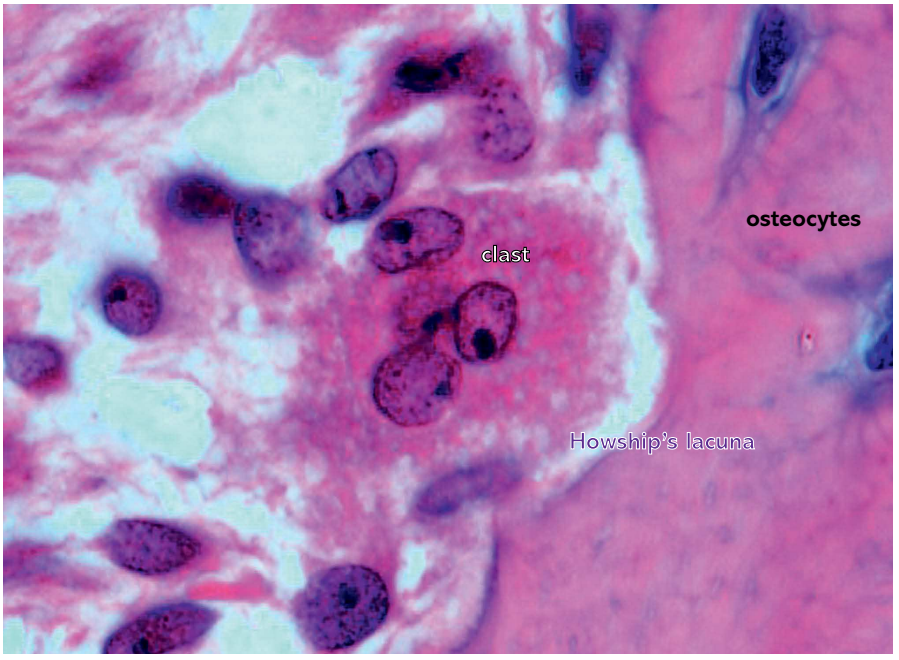

Figure 3 - The network of osteocytes participates in the control of cell function in bone surface as clasts in the context the BMUs. The cytoplasmic processes make contact with surrounding and superficial cells or also act via mediators (HE, 40X).

the mineralized bone matrix of the surrounding region should be resorbed and renewed; preserving them, this phase will not happen and osteogenesis will be able to start soon.

An example of osteocyte preservation can be the divided flap technique in periodontal treatments, which preserves the periosteum attached on the surface. The source of nutrients in the bone are vessels of the periosteum. Preserving the periosteum means to keep alive the osteocytes so that its death does not induce the thin cortical alveolar bone resorption, leading to an undesirable dehiscence or fenestration. Opening the periosteum inevitably leads to the death of the most superficial osteocytes, for they do not receive nutrients from broken vessels during this surgical procedure.

When the osteocytes die in bone remodeling tissue this area will inevitably be reabsorbed. Thus, the osteocytes should be preserved in the bony walls of the cavity prepared earlier to place the implants, avoiding excessive heat or improper manipulation of surfaces, since the death of osteocytes will lead to increased bone resorption at the site, which can disrupt osseointegration.

Probably some orthopedic facial responses can be explained by bone deformities produced. The responses controlled by the osteocytes can change the shape and size of the bone to adapt to new functional demands. This increasingly requires further studies.

The areas close to bone implants remodel themselves constantly, but do not take over the organization and patterns prior to implant placement. The masticatory load on the implants represents stimuli and "aggression" to the bone components, especially to the osteocytes, which capture the surrounding peri-implant deformations as mechanotransductors, releasing mediators that stimulate or inhibit phenomena such as apposition and resorption.

More recently the sclerostin was discovered, a mediator secreted by osteocytes, that circulates the fluid spaces of bone, especially in tubules with cytoplasmic

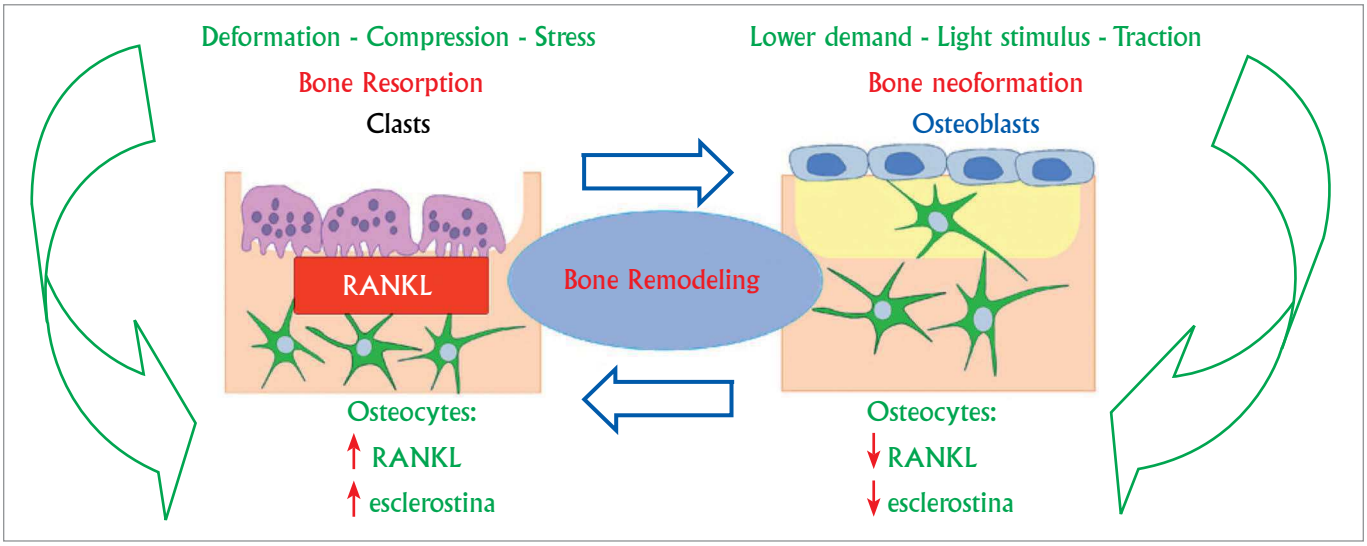

Figure 4 - The osteocytes capture the shape and volume changes increasing or decreasing the release of mediators of the phenomena of bone formation or resorption. Thus, bone remodeling meets the functional demands, changing and adapting structurally (modified de Nakashima et al[14], 2011).

osteocites extensions.[16] It represents a regulatory molecule: If you need more bone, osteocytes release less sclerostin if you need to inhibit bone formation, osteocytes release more sclerostin.

The osteocytes seem to play a central role in bone remodeling.[2] On induced tooth movement there are bone deformations and deflections for each activation devices, especially in the interdental bone crest and free surfaces. When moving a particular tooth to the lingual or buccal, it is known that on the outside, bone is deposited on the cortical surface.[17]

As a true intercommunicating network, stimuli are probably brought to the surface of the outer cortical bone region with periosteal implications by increasing or reducing the thickness of the cortical, which implies in the increase or decrease the bone volume area.

When there is overload on dental implants, excessive forces may induce stress or death of osteocytes. In the periphery of the affected region, probably, osteocytes survivors release mediators that stimulate subjacent and peripheral osteoclastogenesis, such as RANKL, while release more sclerostin to inhibit bone formation at the site. All these peri-implant phenomena can lead to mobility and loss of the implant over time. These increasingly detailed knowledge about bone pathobiology end up enriching and refining surgical techniques and protocols, but especially emphasizing the importance of precise planning in implantology.

These discoveries in bone biology have led to search for new therapeutic alternatives for the bone metabolic problems. Some substances are death inhibitors of osteocytes on the skeleton as a whole and so promote less resorption, for example, estrogens and their modulators, bisphosphonates, calcitonin, CD40 ligand and others.[2] There are still anti-sclerostin to help control bone loss in

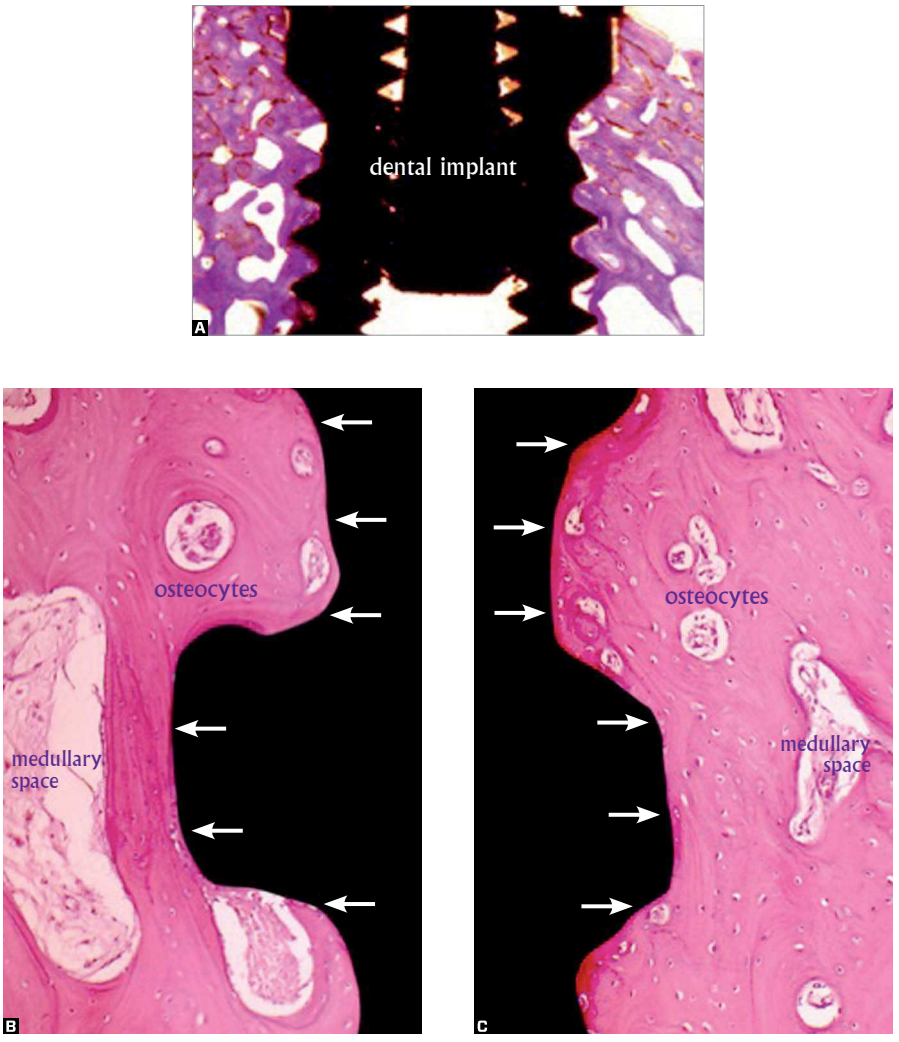

Figure 5 - On the implant surface also is found a network of interconnected osteocytes that capture the forces applied as compressions and deformations due drifts to which the bone is subjected neighbor. The osteocytes mediate phenomena of resorption and bone formation construed in accordance with the functional demands, as appropriate, excessive or minimal (HE; **A** = 10X, **B** e **C** = 25X).

osteopenia and osteoporosis, the most common manifestations of various metabolic bone diseases.

The dental implants are also applied in patients with metabolic bone diseases controlled or undiagnosed, the same way that patients with implant may acquire thi disease during life. One way or another, it is also required that the implantologist know not only the advances of pathobiology bone and its implications for osseointegration, but also its impact on the therapy of metabolic bone diseases.

Conclusions

1. The main targets for the understanding of bone pathobiology used to focus on the osteoblasts and clasts. In recent years, attention has shifted to the osteocytes and their role as bone tissue mechanotransductors. The three-dimensional network formed by interconnections of its extensions can connect a cell with other 20 to 40, something comparable to a neural network.
2. From mediators such as sclerostin and RANKL, osteocytes can greatly influence bone biology, by interfering with the activity of osteoblasts and clasts in trabecular and cortical surfaces. The importance of their role has led many researchers to compare the set of osteocytes to a real endocrine body. When more bone is needed, osteocytes release less sclerostin and when it is necessary to inhibit bone formation, osteocytes release more sclerostin. RANKL, in turn, is connected to local osteoclastogenesis, in order to have more cells capable of reabsorbing the mineralized matrix.
3. New therapeutic ways of controlling the metabolic bone diseases have been targeted at these mediators and their producers: the osteocytes. To study the presence and the specific effects of sclerostin and RANKL in the bone integration can lead to greater detailing of their biological phenomena.

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