

## HOW DO BONE CELLS SENSE MECHANICAL LOADING?

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### ABSTRACT

Influenced by gravity, bone tissue experiences stronger or lighter deformation according to the strength of the activities of daily life. Activities resulting in impact are particularly known to stimulate osteogenesis, thus reducing bone mass loss. Knowing how bone cells recognize the mechanical deformation imposed to the bone and trigger a series of biochemical chain reactions is of crucial importance for the development of therapeutic and preventive practices in orthopaedic activity. There is still a long way to run until we can understand the whole process, but current knowledge has shown a strong progression, with researches being conducted focused on therapies. For a mechanical sign to be transformed into a biological one (mechanotransduction), it must be amplified at cell level by the histological structure of bone tissue, producing tensions in cell membrane proteins (integrins) and changing their spatial structure. Such change activates

bindings between these and the cytoskeleton, producing focal adhesions, where cytoplasmatic proteins are recruited to enable easier biochemical reactions. Focal adhesion kinase (FAK) is the most important one being self-activated when its structure is changed by integrins. Activated FAK triggers a cascade of reactions, resulting in the activation of ERK-1/2 and Akt, which are proteins that, together with FAK, regulate the production of bone mass. Osteocytes are believed to be the mechanosensor cells of the bone and to transmit the mechanical deformation to osteoblasts and osteoclasts. Ionic channels and gap junctions are considered as intercellular communication means for biochemical transmission of a mechanical stimulus. These events occur continuously on bone tissue and regulate bone remodeling.

**Keywords** – *Mechanotransduction cellular; Osteogenesis; Stress mechanical; Weight-bearing; Osteocytes; Osteoblasts; Gap junctions; Ion channels*

### INTRODUCTION

Bone mass maintenance is regulated by various stimuli, which can be grouped into the biochemical (growth factors and hormones) and the mechanical. Regarding the latter, it is known that prolonged immobilization and situations that reduce gravity cause a reduction in bone mass, whereas the impact on bone tissue caused by physical exercise, for example, increases bone mass<sup>(1-10)</sup>.

Regardless of the type of mechanical stimulation (low-power ultrasound, fluid flow, centrifugation, applied static load, vibration, or electromagnetic field), it is recognized by the bone cells after a process called mechanotransduction that is responsible for producing

biochemical reactions from a mechanical (physical) phenomenon, determining a cellular response, which may be bone growth or bone resorption<sup>(8,10-13)</sup>. In this manuscript, we discuss the phenomena and theories about this partially understood area of orthopedics.

#### 1. Amplification of the mechanical stimulus

The primary function of bone tissue is to bear the load of the body. According to Wolff's law cited in Duncan and Turner<sup>(8)</sup>, this tissue is able to adapt to mechanical stresses produced by the weight of the individual and the physical activities that cause deformation of the entire skeleton. Typically, the deformation suffered by bone tissue during locomotion varies from 0.04 to 0.3% and rarely exceeds 0.1%<sup>(14,15)</sup>. Ho-

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wever, in vitro studies have shown that the deformation needed for bone cells to respond to mechanical stimulation is 10 to 100 times greater than that required for bone tissue as a whole (~1-10%). If the same relative deformation (strain) used to stimulate bone cells were used in bone tissue, it would fracture<sup>(8,16)</sup>. This apparent contradiction between stimulation on the macroscopic level and the microscopic (cellular) level was explained and justified by the experimental mathematical model developed by You *et al.*<sup>(16)</sup>, in which the canalicular system in which bone cells (osteocytes) are inserted serves as an amplifier of mechanical deformation generated by physical activity.

### 1.1. Bone histological anatomy

The structure of long bones can be understood schematically as a cylinder, containing within it a number of cylinders, the Haversian canals, which communicate with each other through the Volkmann's canals. The walls that form the Haversian canals are arranged radially and are called lamellae. These consist of bone extracellular matrix (ECM), which consists mainly of hydroxyapatite (inorganic component) and type I collagen (organic component). The bone ECM forms a structure that traps the osteocytes in lacunae within the lamellae. The osteocytes have extensions of their cytoplasm, called cytoplasmic processes (or dendrites), enveloped by canaliculi (Figure 1). Between the canalicular wall and cytoplasmic processes, there is the pericellular space, permeated by a fluid. In the pericellular space there is the pericellular organic matrix (POM) supported by transverse fibrils that anchor and center the cytoplasmic processes of osteocytes in their canaliculi<sup>(16,18)</sup> (Figure 2).

### 1.2. Drag force and shear stress

Put simply, when the bone is deformed, it creates a pressure gradient in the complex system of cylinders, resulting in fluid flow within the pericellular space of cytoplasmic processes, exerting drag force on the POM. The drag force is the result of the frictional force and pressure on a body that moves in a liquid medium. In this case, what moves is the liquid (bone fluid) over the POM, but the drag force is produced in the same way.

The histological structure of bone allows the drag force to produce circular deformations (hoop strains) in the membrane-cytoskeleton system of the cell processes of osteocytes, deformations which are 20 to

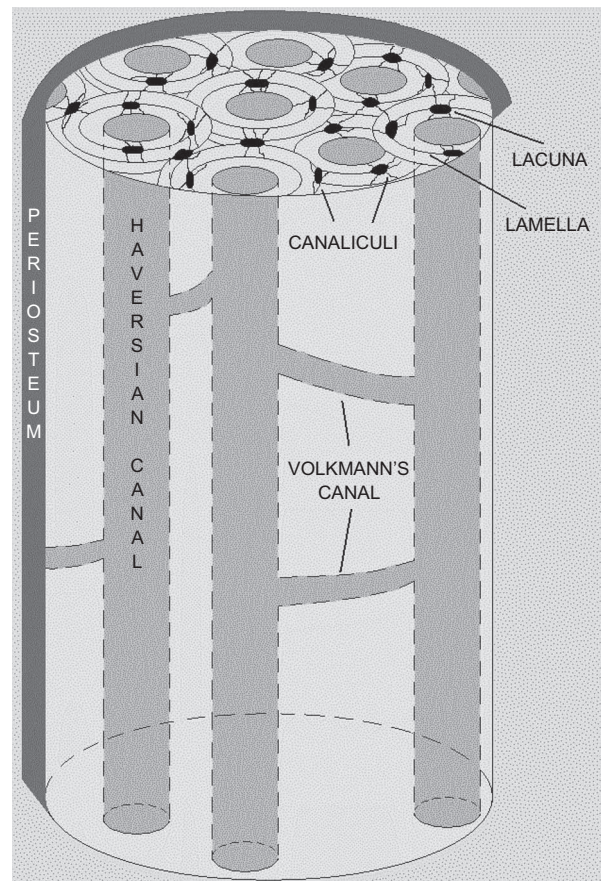


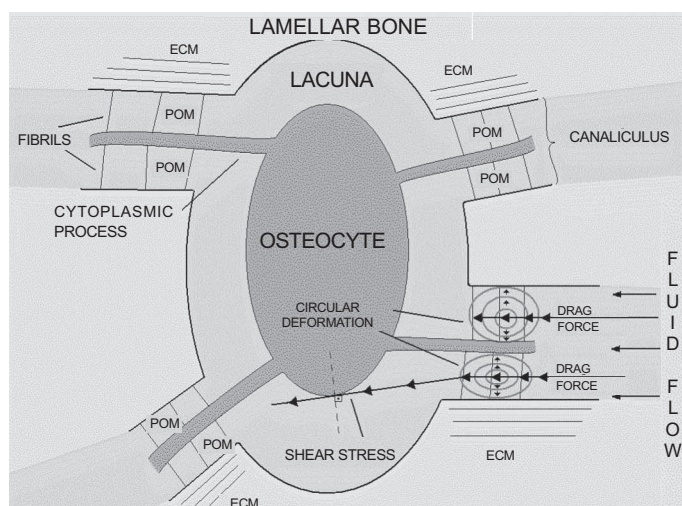
Figure 1 – Bone histological anatomy

100 (or more) times greater than the deformation of bone tissue as a whole. Circular deformations are the normal stresses (i.e., stress that forms a 90° angle to the tangent) to a body with circular symmetry. In other words, circular deformations produce compression and traction forces on the tissue and the greater the magnitude or frequency of the stimulus, the greater the amplification of deformation (Figure 2).

The shear stress (or tangential stress) is the deformation that a body undergoes when subjected to the action of shear (tangential) forces. While You *et al.*<sup>(16)</sup> did not consider shear stress to be responsible for amplifying the deformation of bone tissue at the cellular level, in the view of several authors, its importance should not be ignored<sup>(8,10-12,16,19-21)</sup>. The two types of cell deformation likely contribute to the cellular mechanical stimulus (Figure 2).

## 2. The mechanosensory cell

There are three main types of cells in bone tissue: osteoblasts, osteocytes, and osteoclasts. For decades, osteoblasts and osteoclasts were considered the protagonists of bone remodeling and have therefore been



**Figure 2** – Amplification of the mechanical stimulus. The mechanical deformation of bone tissue produces fluid flow, which, at the level of bone canaliculi, exerts drag force on the cytoplasmic processes and the walls of bone canaliculi. In addition, the fluid flow exerts a tangential force on the plasma membrane of osteocytes, producing shear stress

studied much more than osteocytes, which account for 90-95% of all bone cells. While investigating the behavior of osteocytes, it has been observed that they, like osteoblasts, also respond to mechanical stimulation, displaying the activation of basically the same proteins as osteoblasts<sup>(17,22)</sup>. Since then the question has been: what is the main mechanosensor of bone tissue, the osteoblast or the osteocyte?

Although there is no definitive scientific evidence, it has been widely reported that the osteocytes are the cells that orchestrate bone remodeling<sup>(8,22-24)</sup>, releasing biochemical mediators that regulate the activity of osteoblasts and osteoclasts. In support of this theory, it has been observed that osteocytes produce prostaglandins – mediators of osteoblast and osteoclast activity – faster than the osteoblasts after mechanical stimulation<sup>(25)</sup>, and that osteocytes subjected to fluid flow stimulate the osteogenic activity of osteoblasts<sup>(26)</sup>. It is expected that osteoblasts are sensitive to mechanical stimulation, since osteocytes are derived from these cells. In addition, bone cells are not the only ones capable of mechanotransduction. Fibroblasts<sup>(27)</sup>, chondrocytes<sup>(28)</sup>, cardiomyocytes<sup>(29)</sup>, endothelial cells<sup>(30)</sup>, and rhabdomyocytes<sup>(31)</sup> also have this capability. However, for the scientific community, it is more logical that osteocytes are more utilized as mechanosensors in bone tissue than osteoblasts because they are part of the canalicular system of mechanical deformation amplification, while osteoblasts are located on the periphery of the bone tissue, in the periosteum.

### 3. Piezoelectricity

The piezoelectric effect is a biological response to mechanical stimulation that has been known for a long time, documented by Fukada and Yasuda in 1957 after observing the production of a negative electrical charge in areas of bone compression and a positive charge in the areas of traction<sup>(32)</sup>. This effect was reproduced and measured by other authors, such as Butcher *et al.*<sup>(33)</sup> and Qin *et al.*<sup>(34)</sup>. The hypothesis for this phenomenon was reported by Duncan and Turner<sup>(8)</sup>, who believed that the fluid flow generated by mechanical stresses produced power currents (streaming potentials), modulating the cellular response. Currently, it is known that the piezoelectric effect is not mechanotransduction: it is only a marker of fluid flow. This causes the activation of mechanosensitive ion channels, especially potassium and calcium ion channels, inducing ion flux in bone cells, resulting in a change in the cell membrane potential, which may be positive (depolarization) or negative (hyperpolarization). The bone cell can quickly identify the characteristics of mechanical stimuli and respond electrophysiologically in different ways, with varying degrees of ion channel activation, resulting in the hyperpolarization or depolarization of the plasma membrane. What determines the intensity of the activation of ion channels is unclear, but it is known that the intensity and frequency of mechanical stimulation, as well as the velocity of fluid flow, regulate the activation of these channels. It is known that hyperpolarization is associated with osteogenesis, and depolarization with bone resorption. In addition to regulating the cellular response to mechanical stimulation, it is believed that activation of ion channels biochemically transmits the mechanical deformation to neighboring osteocytes, osteoblasts, and osteoclasts<sup>(12,33-39)</sup>.

### 4. Mechanotransduction

Mechanotransduction can be interpreted as the process of producing a biochemical reaction from a mechanical stimulus. The biochemical chain reactions induced by mechanical stimuli act at the cellular level and can cause inhibition of apoptosis, increased cell proliferation, altered cell migration, among other effects.

When an external mechanical stimulus deforms bone tissue, circular deformation and shear stress occur at the cellular level, acting on the plasma membrane of osteocytes, and are transmitted throughout

the cell through a complex network that connects the plasma membrane to the cell nucleus, called the integrin-cytoskeleton-nucleus extracellular (and pericellular) matrix system. Duncan and Turner<sup>(8)</sup> devised a model of mechanotransduction in which this system is critical; the mechanical stress causes strain between the constituents of this system, allowing for the transmission of deformation from the ECM to the nucleus of cells. Elaborating on this model in light of current knowledge, the authors of this review believe that its operation is like a lever system with multiple pivot points, which represent the interactions between the molecules that comprise the extracellular/pericellular matrix (collagen, fibronectin, and fibrils), the different subunits of integrins, the cytoskeleton (actin, vinculin, talin, paxillin, and  $\alpha$ -actinin), and the nuclear membrane<sup>(8,17,18)</sup>. Therefore, the pivot points can be tailored to the type and quantity of molecules interacting with each other, which allows for various types of transmission of mechanical deformation.

#### 4.1. Integrins

In each region of the ECM/POM-integrin-cytoskeleton-nucleus system, proteins are interacting with their constituents. The integrin region is the most understood, and is considered the most important for mechanotransduction. The name integrin refers to its function of integrating the interior of the cell (cytoskeleton) to its exterior (ECM and POM). Integrins are heterodimeric transmembrane cell adhesion glycoproteins composed of  $\alpha$  and  $\beta$  subunits. In humans, there are 24 well-established types of integrins, resulting from combinations of the 18 different  $\alpha$  subunits and eight types of  $\beta$  subunits. The different combinations of  $\alpha$  and  $\beta$  subunits determine the specificity of integrin binding to ECM components and of the cytoskeleton and cytoplasmic proteins, as well as the degree of affinity for each ligand. Osteoblasts express the subunits  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha v$ ,  $\alpha 6$ ,  $\beta 1$ ,  $\beta 3$ , and  $\beta 5$  *in vitro*<sup>(40,41)</sup>, whereas *in vivo*, the expression of integrins is restricted primarily to  $\alpha 3$ ,  $\alpha 5$ ,  $\alpha v$ ,  $\beta 1$ , and  $\beta 3$ <sup>(42)</sup>. In these cells, the subunits that are known to respond to mechanical stimuli are  $\alpha 2$ ,  $\alpha 5$ ,  $\beta 1$ , and  $\beta 3$ , and the heterodimers that have been demonstrably involved in mechanotransduction are  $\alpha 5\beta 1$  and  $\alpha v\beta 3$ .

It is believed that after the mechanical deformation of the plasma membrane, an integrin conformational change occurs, creating high-affinity sites for chemi-

cal reactions in its chemical structure resulting in connections with other integrins and components of the cytoskeleton. In other words, mechanical stimulation functions as an enzyme that catalyzes a biochemical reaction. Links between various integrins form clusters that increase the avidity of these proteins to bind to other molecules. The clusters of integrins anchor to components of the cytoskeleton, inducing its remodeling, forming a specialized structure called focal adhesion (or focal contact). This structure is dynamic, as it forms in response to mechanical stimulation and breaks down in response to the absence of that stimulus. It is located near the plasma membrane and the ECM, and it recruits several molecules involved in mechanotransduction: tyrosine kinases, ion channels, phospholipase C, and mitogen-activated protein kinase (MAPK), among others<sup>(11,12,43-46)</sup>.

#### 4.2. Focal adhesion kinase (FAK) pathways

Of all the molecules that interact with integrins in focal adhesions, the one that is most studied and considered essential in the conversion of mechanical phenomena to biochemical phenomena is focal adhesion kinase (FAK), an adapter protein of the tyrosine kinase group, the structure of which allows it to interact with several proteins, allowing for the formation of multiple protein complexes. This feature can increase cellular response if the activity of the multiple complexes is additive, or can induce different types of cellular response if the activity of the different complexes is on different paths.

It has not been well established whether FAK is always connected to integrins, or whether it is recruited by integrins when they form the focal adhesions. The authors of this manuscript believe that the two forms exist and have their roles. After the deformation of integrins, the FAK must subsequently be deformed, also undergoing changes in its structure, resulting in autophosphorylation at tyrosine residue 397 (Tyr-397). This activates FAK, with the creation of a high-affinity site for binding to proteins containing the SH2 domain (Src-homology-2), such as Src and the p85 subunit of phosphatidylinositol 3-kinase (PI3K). In response, the activated FAK combines with Src or with PI3K, activating FAK-Src/Grb2/Sos/Ras-Raf/MEK/ERK-1/2 and FAK/PI3K/Akt/NF- $\kappa$ B pathways, respectively. The extracellular signal-regulated kinase-1/2 (ERK-1/2) and Akt are

effector proteins of these pathways and may exercise their functions in both the cytoplasm and the nucleus of bone cells. What determines whether these proteins, including FAK, are in the nucleus or cytoplasm is not well established, but research suggests that this is determined by the amount of protein and the amount of activated protein. It has been speculated that nuclear localization involves the increased effects of these proteins (FAK, ERK-1/2 and Akt), which may be: induction of migration, cell proliferation and differentiation, and inhibition of apoptosis<sup>(8,10,20,30,47-54)</sup> (Figure 3).

#### 4.3. Biochemical transmission of the mechanical stimulus to other cells

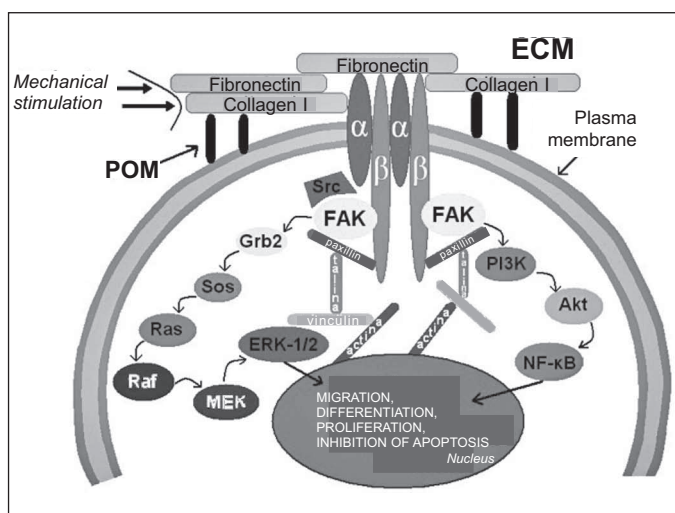
Even the cells that are not deformed by mechanical force exhibit a biochemical response similar to that of those that were deformed. In these cases, the phosphorylation (activation) of connexin 43 was observed, which is responsible for activating the gap junctions, the intercellular communication structures located in the cytoplasmic processes of the osteocytes. Gap junctions allow for the exchange of ions and small molecules, particularly prostaglandin E2 (PGE<sub>2</sub>), between osteocytes and between osteocytes and osteoblasts.

The activation of ion channels should also contribute to the biochemical transmission of mechanical stimulation. The mechanical deformation causes activation and inactivation of ion channels, especially calcium and potassium channels, which generates a negative action potential, with membrane hyperpolarization, which is transmitted to neighboring cells, activating intracellular reactions<sup>(26,35,37,55,56)</sup>.

Although intriguing, this subject has gone largely unexplored, with current research focused primarily on the integrin pathway.

#### 4.4. In vivo mechanotransduction

Most studies have been based on in vitro experimental models<sup>(11,16,19,21,23,25,26,35,46)</sup>. Despite the undeniable contribution of these studies in guiding subsequent research, the limitations of an in vitro study when compared with reality, that is, an in vivo study, should be considered<sup>(57,58)</sup>. The beneficial effect of low-power ultrasound (lpUS) in accelerating bone healing in bones with some type of injury has been documented<sup>(59-66)</sup>.



**Figure 3** – FAK-dependent mechanical stimulus signaling in bone cells. The FAK maintains a close relationship with the  $\alpha$  and  $\beta$  subunits of integrins and cytoskeletal components (actin, paxillin, talin, and vinculin), which are also in contact with the nucleus

The authors of this review have developed a rat model to assess the “molecular” effect of 20 minutes of daily treatment with lpUS in the intact tibias of these animals. The tibias were stimulated for at least one week. After treatment, bone proteins involved in mechanotransduction (FAK, ERK-1/2, and Akt) were measured. It was observed that long-term treatment with lpUS increased synthesis of FAK and ERK-1/2 in a non-cumulative manner, that is, at a certain point, the synthesis of these proteins stopped increasing, as if something had blocked the effects of lpUS. The activation of FAK, ERK-1/2, and Akt occurred early and, at times, was sustained for 15 hours (only FAK and ERK-1/2), unlike what occurs when applying a single stimulus. The finding of a greater amount of insulin receptor substrate-1 (IRS-1), a protein activated by growth factors and hormones in one of the stimulated groups, suggests that the lpUS interferes with cellular reactions mediated by growth factors, which is a controversial issue in in vitro models that has been little investigated. In addition, increasing the amount and activation of FAK in the bones of animals stimulated with the lpUS equipment turned “off”, although lower than in animals stimulated with the equipment turned on, indicates that “muscular” stress interferes with the activity of mechanosensitive proteins in “bone” tissue<sup>(67,68)</sup>.

## FINAL CONSIDERATIONS

Mechanotransduction is a phenomenon that is constantly occurring in bone tissue, with important clinical implications that should be acknowledged. However, because it is a complex phenomenon that involves se-

veral types of molecules arranged in multiple systems that interact with each other, it is still poorly understood. The detailed study of all the phases involved in the chain of molecular events is essential to understanding its entirety and to act systematically in this process, which apparently remains very complex.

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