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BONE MECHANOTRANSDUCTION: A REVIEW

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Abstract- This review focus on the bone physiology and mechanotransduction elements and mechanisms. Bone biology and architecture is deeply related to the mechanical environment. Orthopaedic implants cause profound changes in the biomechanics and electrophysiology of the skeleton. In the context of biomedical engineering, a deep reflexion on bone physiology and electromechanics is needed. Strategic development of new biomaterials and devices that respect and promote continuity with bone structure could have a major impact on patient's well being.
Keywords– Bone, Mechanotransduction, Electromechanics, Piezolectricity

Introduction

Bone is a complex and dynamic tissue, both light and strong, and that provides structural support for the body, protection of internal organs and acts as levers to which muscles are attached, allowing movement. Besides these major functions, the skeleton is also essential as mineral reservoir and participates in acid-base balance.

Bone is composed of 70% inorganic component (of which 95% is hydroxyapatite and 5% impurities impregnated in hydroxyapatite), 22% to 25% of organic component (of which 94-98% is mainly collagen type I and other non-collagen proteins and 2%-5% are cells) and 5 to 8% is water.

Bone mechanical properties depend on mineralization degree, porosity, composition and organization of solid matrix. Therefore, the mechanical behaviour of a whole bone is highly dependent on its properties at a microscale [2]. Both cancellous [**Fig.(1)**] and compact [**Fig.(2)**] bone show anisotropic behaviour, i.e. the Young's modulus depends on the direction of the load, due to the deliberate direction of lamellae [3,4]. In long bones, fundamental for load bearing and leverage, stiffness along the long axis was favoured. Vertebral bodies function like shock absorbers and flexibility was preferred and achieved through the cancellous porous architecture [5].

Long bones, as other natural composite tubular structures, combine great strength and fatigue resistance against axial compression forces with minimum weight. However, much of the strain measured in bone is due also to bending moments [1]. Normal loading of long bones combines compressive and bending efforts, causing in humans a large variation of strains, up to 400 to 2000 µstrains or even as high as 4000 µstrains [1,6,7]. The dynamic process of bone resorption and formation occurs on both cortical and trabecular bone and it occurs in response to mechanical loading, calcium serum levels and a wide range of paracrine and endocrine factors. Strain magnitude, frequency and loading duration influence bone remodelling.

In the context of biomedical engineering, a deep reflexion on bone physiology and electromechanics is needed. Orthopaedic implants cause profound changes in the biomechanics and electrophysiology of the skeleton. Strategic development of new biomaterials and devices that respect and promote continuity with bone structure could have a major impact on patient's well being.

This review is focused on the bone physiology, mechanotransduction elements and mechanisms.

Bone Cells

The cell population in mature bone consists of essentially three types: osteoblasts, osteocytes and osteoclasts.

Osteoblasts are derived from mesenchymal cells, sharing a common heritage with chondrocytes, myoblasts and fibroblasts. Osteoblast differentiation depends on osteotropic hormones and cytokines but also on the mechanical microenvironment [8].

Osteoblasts are typically round, with morphological characteristics that are in agreement with their secretory functions. During the process of matrix production and early mineralization, they present a very prominent Golgi complex, with multiple vesicles and vacuoles that are thought to contain pro-collagen and proteoglycans [9].

Osteoblasts can also remain on quiescent bone surfaces as bone lining cells, flat and with few cell organelles. During the process of maturation osteoblasts show increasing levels of expression of pro-collagen, osteocalcin and osteopontin [**Fig.(3)**]; bone sialoprotein seems to be more strongly expressed at intermediate phases of differentiation [10-11]. Gap junctions' inhibitors impair osteoblast differentiation so these channels of communication to neighbouring cells seem essential for osteoblast maturation [12]. Osteoblasts produce osteoid that becomes progressively mineralized. As mineralization occurs, osteoblasts are trapped within the mineralized matrix and become osteocytes. Osteocytes are therefore terminally differentiated osteoblasts embedded in the osteocytic lacunae. Lacunae are roughly 10 µm in length, placed between lamellae and connected to neighbouring lacunae by a system of canaliculi [**Fig. (4)**]. Osteocytes have long cell processes that run through the canaliculi. The tips of these dendritic processes allow osteocytes to maintain direct contact and to connect to overlaying osteoblasts and bone lining cells, through gap junctions [4, 13-14]. The resulting functional syncytium shares a common environment, proteoglycans and extracellular fluid [15].

Osteocyte functions probably include maintaining bony matrix and acting as mechanosensors [16, 17]. It has been hypothesized that sensation of electrical signals is one of the functions of osteocytes, and that the electrical signals mediated by osteocytes may regulate the overall behaviours of cells in bone tissue, including coupling effect of osteoblasts and osteoclasts [18].

Osteoclasts are multinucleated cells, sharing the same lineage as macrophages and monocytes [**Fig.(5)**]. Osteoclasts originate from the hematopoietic stem cells (HSC) and share with macrophages the ability to merge, forming multinucleated cells, and to phagocytise [19]. The promyeloid cell precursor is able to differentiate into either an osteoclast or a macrophage and the differentiation pathway depends on whether the precursor cell is exposed to a receptor activator of various ligands (RANKL, osteoprotegerin and osteoclast differentiation factor - ODF) or to colony-stimulating factors, and closely intertwined with the immune system [20-22]. The bone resorption process begins with differentiation and recruitment of osteoclast precursors, which fuse to form mature multinucleated bone-resorbing osteoclasts.

The Bone Matrix

As already stated above, the mineral phase is the most part of bone. Hydroxyapatite nanocrystals coat the collagen network.

Collagen is essential for maintenance of structure and biomechanical properties of bone. Type I collagen is the most abundant type of collagen and is widely distributed in almost all connective tissues with the exception of hyaline cartilage, and is the major protein in bone, comprising about 80% of the total proteins present in bone. Collagen I is composed of a triple helix formed by the combination of three long peptide sequences. After synthesis, and whilst still inside the osteoblast, collagen goes through a series of enzymatic modifications, namely by the addition of hydroxyl groups to the proline and lysine residues [23]. Once collagen leaves the cell further cross-linking within and between collagen molecules occurs. Mutations in collagen chain genes lead to disease such as osteogenesis imperfecta [23, 24]. The triple helical tropocollagen units are aligned in fibrils that display permanent dipole moment. So, collagen behaves as a piezoelectric and piroelectric material, and can act as an electromechanical transducer [25, 26]. The piezoelectric properties of collagen and the innate polarity of the molecules are associated with the mineralization process, as cell-free *in vitro* studies suggest, for under compression, negative charges on the collagen surface are exposed and attract calcium cations, quickly followed by phosphate ions [26, 27].

Although present in much smaller amounts, non-collagenous proteins are also essential for normal bone function and mechanical properties.

Fibronectin is synthesized by both osteoblast precursor cells and mature bone cells; it can also be produced at distant sites such as the liver and enter systemic circulation. Recent studies suggest although osteoblast secreted fibronectin influences osteoblast functions, only circulating fibronectin exerts effects on the bone matrix [23, 28]. Fibronectin binds to collagen and may act as an extracellular scaffold that binds and facilitates interactions of bone morphogenetic protein type 1 (BMP1) with substrates that include procollagen and biglycan [29].

Osteonectin is another non-collagenous protein present in bone matrix that has a strong affinity to collagen and mineral content; osteonectin knockout mice suffer from osteopenia resulting from low bone turn-over, with defective function of both osteoblasts and osteoclasts. Another matrix protein, thrombospondin-2, also exerts its effects on osteoblast proliferation and function [30, 31].

Osteopontin (OPN) is a multifunctional non-collagenous glycoprotein present in bone matrix, expressed in various degrees by proliferating pre-osteoblasts, osteoblasts and osteocytes but also by fibroblasts, osteoclasts and macrophages [32, 33]. OPN production is known to be increased in association with mechanical loading [33, 34], and its deficiency significantly decreases bone fracture toughness, leading to heterogeneous mineral distribution, since OPN is known to bind strongly to hydroxyapatite, as well as to cell surface [35, 36].

Bone sialoprotein (BSP) is a highly glycosylated and sulphated phosphoprotein that is found almost exclusively in mineralized connective tissues. Characteristically, polyglutamic acid and arginine-glycine-aspartate (RGD) motifs, able to bind hydroxyapatite and cell-surface integrins, respectively, have been conserved in the protein sequence [37]. Bone sialoprotein knockout mice have smaller size and weight but they are viable and able to breed normally; they present reduced amounts of cortical bone, higher trabecular bone mass with very low turn-over. Response

to mechanical unloading is maintained in BSP defective mice, as opposite to OPN knockout mice [38].

Bone proteoglycan (PG) structure and localization is varied (pericellular and extracellular in the organic bone matrix) and reflects a wide spectrum of biological functions within a unique tissue. PGs play important roles in organizing the bone extracellular matrix, helping to structure the tissue itself as active regulators of collagen fibrillogenesis. PGs also display discerning patterns of reactivity with several constituents including cytokines and growth factors, such as transforming growth factor-β (TGF-β) or osteoprotegerin thereby modulating their bioavailability and biological activity in the bone tissue [39].

Bone Mechanotransduction: The Key Elements

How each individual cell, either osteoblast or osteocyte, senses and responds to mechanical loading is only starting to be understood. The transduction elements include extracellular matrix (ECM), cell-cell adhesions, cell-ECM adhesions, membrane components, specialized surface processes, nuclear structures and cytoskeleton filaments.

Membrane associated mechanotransduction mechanisms rely on the properties of the phospholipid bilayer. Mechanotransduction pathways are disrupted in bone cells if there is depletion of cholesterol, inhibiting the response to hydrostatic and fluid shear stress [40]. Actin polymerization and assembly is influenced by membrane cholesterol levels, as indicated by studies that report stress fibber formation after acute cholesterol depletion [41, 42]. But even with an intact membrane, if integrin binding is disturbed, actin cytoskeleton reorganization in response to shear stress will not occur [43]. The integrins are a superfamily of cell adhesion receptors that bind to extracellular matrix ligands, cell-surface ligands, and soluble ligands. Integrins possess an extracellular portion with several domains that may link to large multi-adhesive ECM molecules, which in addition to binding integrins, also bind to other ECM molecules, growth factors, cytokines and matrix-degrading proteases [44]. Their first recognized function was bridging the ECM and the cytoskeleton. Many integrins link to the actin cytoskeleton, but connection to the intermediate filament network also occurs [45]. Integrins act as bi-directional signalling receptors involved in outside-in and inside-out signalling. The inside-out signalling mainly acts to bring the integrin extracellular domains into the active conformation. In the outside-in pathway, upon binding of ECM proteins, occurs receptor clustering and redistribution of cytoskeletal and signalling molecules into focal adhesions at the sites of cell-ECM contact [46, 47]. Osteocytes are highly specialized in their interaction with ECM; osteocyte cell bodies express β1 integrins while cell processes express β3 integrins, the latter in a punctuate distribution similar to matrix attachment sites but involving far fewer integrins. These specializations are likely to have physiological consequences for mechanosensitivity [48].

Apart from integrin, other transmembrane proteins are responsible for conduction of mechanical stimuli; cadherins mediate force-induced calcium influx [49, 50]. In osteoblasts mechanical load applied to β1-integrin subunit also results in calcium influx [51] but the rise in cytosolic calcium is independent from gap junctions, where another type of protein, conexins, play a major role [52].

Gap junctions are transmembrane channels that connect the cytoplasm of neighbouring cells. Small metabolites, ions and signalling molecules like calcium and cAMP pass through these channels, but molecular weight must be lower than 1 kD_a [53, 54]. Gap junctions are formed by connexins and in bone tissues, three types were identified: Cx43, Cx45 and Cx46 [14]. In osteoblastic MCT3C3 and ROS 17/2.8 cells with intact gap junctions fluid flow induces PGE_2 production, absent if gap junctions are disrupted [52, 55]. Mechanical loading increases the expression of connexin 43 mRNA in osteoblasts and bone lining cells in mice alveolar bone [56].

Specialized surface cell structures may also be responsible for the detection of mechanical stimuli. Primary cilia were described in osteoblasts and osteoblast-like cells, originated in the centrosome and projecting from the surface of bone cells [57]. Its deflection during flow indicates that they have the potential to sense fluid flow. These cilia deflect upon application of 0.03 Pa steady fluid flow and recoil after cessation of flow [58, 59]. Calcium influx occurs is osteoblasts in response to oscillatory fluid flow [52] but, unlike for kidney epithelial cells, primary cilia in bone cells translate fluid flow into cellular responses independently of Ca^2 flux and stretch-activated ion channels [59].

The cytoskeleton is also a key element in cell mechanotransduction. The transfer of forces across the network of microfilaments, microtubules and cell adhesions allows focused stresses applied on the surface membrane to exert effects at distant sites such as mitochondria and nucleus and the plasma membrane on the opposite side of the cell. The transmission of tension towards the ECM promotes structural changes that add strength to the tissue at a higher organization level [60, 61]. Specific transmembrane receptors such as integrins couple their cytoskeleton network to the ECM. Integrins connect to the cytoskeleton through focal adhesions that contain actin-associated proteins such as talin, vinculin, paxilin and zyxin. There is evidence that mechanical properties of the ECM affect the behaviour of cells from osteoblast lineage, with mature focal adhesions and a more organized actin cytoskeleton associated with more rigid substrates, suggesting that tuning substrate compliance may enable control over differentiation [62]. The biochemical nature of the substrate, its rigidity and spatial organization are recognized by cells through signalling from molecular complexes that are integrin-based. Although cells show immediate viscoelastic response of individual focal adhesions, the deformation of a cell in consequence of

an applied stress does not correspond to the predicted strain of a homogeneous viscoelastic material; the interior of the cell, and thus the cytoskeleton, is anisotropic. Due to the complex network of microtubules and microfilaments and the way this network spreads and is connected to the point of applied force, it is possible that structures far away from this point are displaced further than closer ones, as it is possible to record displacements towards the origin of the compressive stimulus; the anisotropic character of the constitutive mechanical properties (elastic modulus, shear modulus) allows cells to respond to an external force accordingly to its magnitude and direction [63-65]. Displacement of organelles within the cell subjected to external forces is closely dependent on the actin and tubulin network, namely for the translation of applied forces into mitochondrial movements. Since mitochondria are semi-autonomous organelles, highly dynamic, it is likely that the perturbation caused by mechanical stimulus exerts biological effects on their function [64].

Osteoblasts, osteoid-osteocytes and mature osteocytes have different mechanical properties. The elastic modulus is higher on the cell peripheral area than in the nuclear region; as bone cells mature, the elastic modulus decreases, both in the peripheral and nuclear regions. When mechanical stimulus (local deformation) is applied on microparticles attached to osteocyte cell body and processes, different sensitivity levels are found. A much smaller displacement of the microparticles attached to the processes is needed to cause an intracellular calcium transient that rapidly propagates to the cell body. If local stimulus is applied to the cell body, the reaction is slower and a higher displacement is needed to elicit the calcium transient. These findings might relate with differential localization of the actin filaments and the distance to fixed points (integrin mediated focal adhesions), helping to understand the mechanosensing mechanisms of osteocytes [66]. Furthermore, focal adhesion area is smaller in mature osteocytes, when comparing to osteoblasts. If peptides containing Arginine-Glycine-Aspartic acid (RGD) sequence are added to culture medium, both the focal adhesion area and the elastic modulus of osteoblasts decrease whilst osteocytes remain unaffected [67].

Mechanotransduction Mechanisms

The question of how cells convert mechanical responses into biochemical responses is only partially answered. Some of the messenger molecules, like nitric oxide, are produced as a consequence of mechanical stimuli and have been thoroughly studied but they perform a variety of functions, so much is yet to be known about how responses are orchestrated in the context of the living organism. Recent models suggest mechanotransduction may be carried out also by solid-state mechanochemistry mechanisms [68].

Strain magnitude, frequency and loading duration influence bone remodelling. Wolff defined the mathematical equations that allowed prediction of trabeculae orientation and thickness [69]. According to Turner, bone remodelling is determined by dynamic loading - short periods of loading quickly trigger a response; prolonging loading times any further diminishes the magnitude of bone cell response; bone cells also accommodate to routine loading, diminishing the amplitude of the answer triggered by a same repeated stimulus [70]. These theoretical rules are supported by several studies. In *in vivo* studies, increasing loading frequency decreased the threshold for osteogenesis and increased strain-related bone deposition [71]. Cortical bone adaptation is nonlinear when it comes to frequency response; under loads varying from 1 to 2 N, and frequencies of 1, 5, 10, 20 and 30 Hz, the changes in geometry were more significant with increasing load frequency, with a plateau for frequencies beyond 10 Hz [72]. Strain magnitude also influences bone formation [73], along with the number of loading cycles at low frequencies [74]. Strain distribution seems to condition the skeletal adaptation also. Unusual strain distribution will quickly trigger an osteogenic response, as suggested by the extensive periosteal and endosteal bone proliferation described by Rubin & Lanyon in a study conducted in roosters [75]. Rest periods in between loading cycles also intensify osteogenic response [76].

There is evidence that other mechanisms, apart from direct deformation of cells, are involved in bone cells mechanical stimulation. Although peak strains may be considerable high in long bones during strenuous exercise, there are studies showing that strains as low as 0.15% are enough to ensure osteoblast recruitment *in vivo* [75]. Because of the complex architectural structure of bone, it is difficult to accurately measure the actual deformation of each cell individually. However, it is known that when the canalicular, porous structure of bone is mechanically loaded, fluid flows along its structure, carrying electrically charged particles [77]. The deformation of the fixed-charged matrix causes a fluid flow relatively to the solid matrix. This is a phenomenon common to biological tissues. A thin layer of fluid with particles with opposite charge to that of the matrix and bone cells is formed; when a non-uniform mechanical load is applied to the bone structure, the freely moving ions in the fluid move away from the matrix. The movement of the electrical charged fluid creates an electrical field collinear to the fluid flow. This results in the generation of an electrical potential and the phenomenon is known as strain generated bone streaming potential [77-79]. Most interesting is the fact that the density of matrix fixed charges influences the magnitude of the streaming potential [80]. Different matrix charge density may, therefore, alter the mechanical sensitiveness along the bone and, indirectly, influence dynamic stiffness.

Fukada and Yasuda were the first to describe bone piezoelectrical properties, in the 50s. When submitting dry bone samples to compressive load, an electrical potential was generated, as explained by the direct piezoelectric effect [81]. The nature of the piezoelectric

effect is closely related to the occurrence of electric dipole moments in solids. In connective tissues such as bone, skin, tendon and dentine, the dipole moments are probably related to the collagen fibbers, composed by aligned strongly polar protein molecules [25, 82, 83]. The architecture of bone itself, with its aligned concentric lamellae, concurs for the existence of potentials along bone structure [82].

Bone piezoelectric constants, i.e. the polarization generated per unit of mechanical stress, change according to moisture content, maturation state (immature bone has lower piezoelectric constants when comparing to mature bone) and architectural organization (samples from osteossarcoma areas show lower values) [84]. In dentin, piezoelectric constants rise as moisture contents increases but, most interestingly, they behave in an anisotropic fashion; tubule orientation strongly influences piezoelectricity [85]. Early studies concentrated on dry bone and because collagen's piezoelectricity was described as nearly zero with 45% moisture content, there were doubts that wet bone could, in fact, behave as a piezoelectrical material, but further studies confirmed it in fact does [81, 84, 86]. Some of the published studies reinforce the importance of fluid flow as the main mechanism for stress generated potentials in bone, and piezoelectricity's role was, and still is, quite unknown [87].

More recently, bone piezoelectrical properties have rouse interest, in the context of bone physiology and electro-mechanics. It has been related to bone remodeling mechanisms, and to streaming potential mechanisms [88, 89]. Piezoelectricity explains why, when under compression, collagen reorganizes its dipole and shows negative charges on the surface, which attract cations like calcium. Conversely, if tensed, collagen yields predominance of positive charges, thus obviously influencing the streaming potential and mineralization [26, 27].

Conclusions

Taking into account the present knowledge on bone physiology, developing materials for bone regeneration able to respect bone electrophysiology seems like a logical move whenever treatment of bone defects is being considered. The commercially available biomaterials for bone replacement and reinforcement don't take into account the bone natural piezoelectricity and the mechanism of streaming potential. So, besides the ever present considerations on implants' mechanical properties and chemical composition, reflecting on these aspects may point out new directions and improvement of clinical results.

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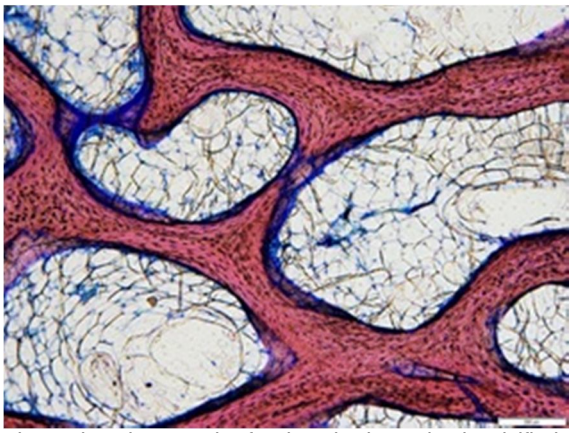

Fig.1 Microphotograph of trabecular bone (undecalcified bone section of sheep tibia, Giemsa-Eosin). The picture illustrates the sponge-like structure of cancelous bone, formed by the intertwining of trabeculae.

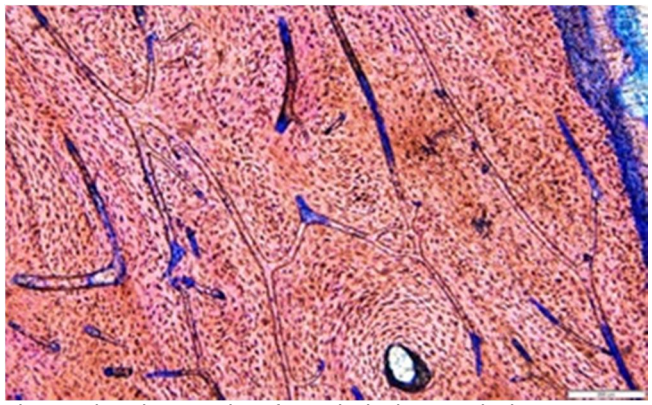

Fig.2 Microphotograph of cortical (compact) bone (undecalcified bone section of sheep femur, Giemsa-Eosin). The vascular network and an osteon, organized around the Havers' channel may be observed.

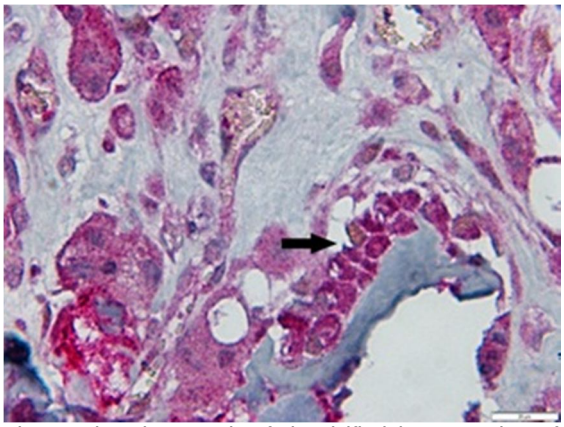

Fig. 3 Microphotograph of decalcified bone section of sheep femur, double Fast-Red and DAB immunohistochemistry staining for osteopontin and PCNA. Arrow signals osteoblasts expressing osteopontin.

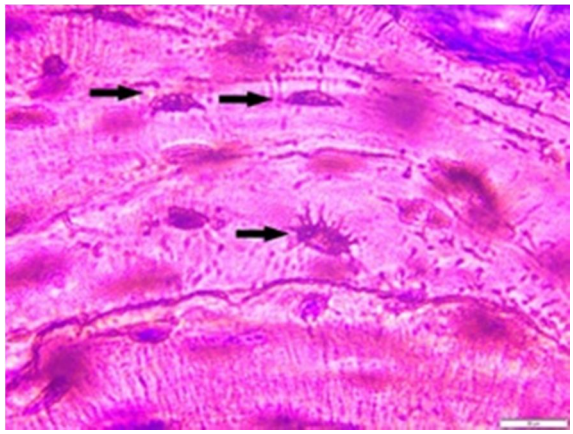

Fig.4 Microphotograph of undecalcified bone section of sheep femur, Giemsa-Eosin. Arrows signal osteocytes. Some of the canaliculi where cell processes run are evident.

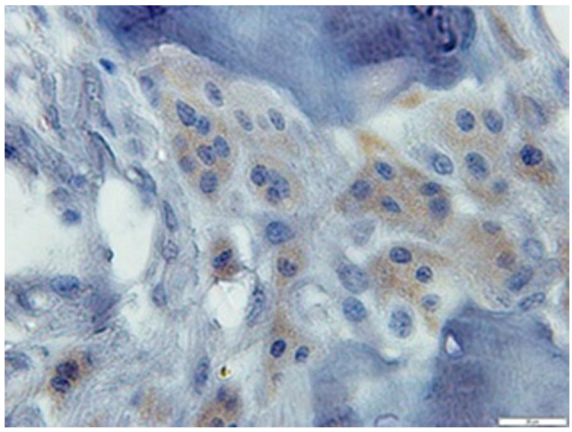

Fig.5 Microphotograph of decalcified bone section of sheep femur, DAB immunohistochemistry staining for tartrate-resistant acid phosphatase (TRAP); positive stained osteoclasts are evident on bone surface (stained brown).