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Cells in focus

The osteocyte

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Abstract

Osteocytes are the most numerous cells in mature bone and have the potential to live as long as the organism itself. However, study and subsequent understanding of osteocyte biology has been thwarted by the remote location of the cell in the mineralized matrix. This review is intended to synthesize current understanding of osteocyte biology and to suggest future paths that will promote understanding of this obscure cell and translation of knowledge to disease prophylaxis and management.

Keywords: Bone; Mechanotransduction; Pathoanatomy; Fluid flow; Osteoporosis

Cell facts

- most abundant cells in mature bone;
- average half-life of 25 years;
- do not divide;
- periosteocytic space provides largest surface area for ion exchange and molecular filtration in body.

1. Introduction

Bone provides mechanical support for the organism, a site for haemopoiesis, a reservoir for calcium storage, and a mobilization surface for ion homeostasis, so preservation of bone health is a critical for survival. Osteocytes, the most abundant cells in mature bone, have a putative role in mechanotransduction that is assumed to modulate activity associated with remodeling and bone turnover. Recent studies underscore the importance of osteocyte viability in maintenance of bone tissue health, and implicate bone remodeling and osteolysis as processes that insure viability for cells, i.e. osteocytes, trapped in a relatively impermeable, mineralized matrix.

2. Cell origin and differentiation

Due to the difficulty in observing osteocytes in situ, current understanding of osteocyte biology has

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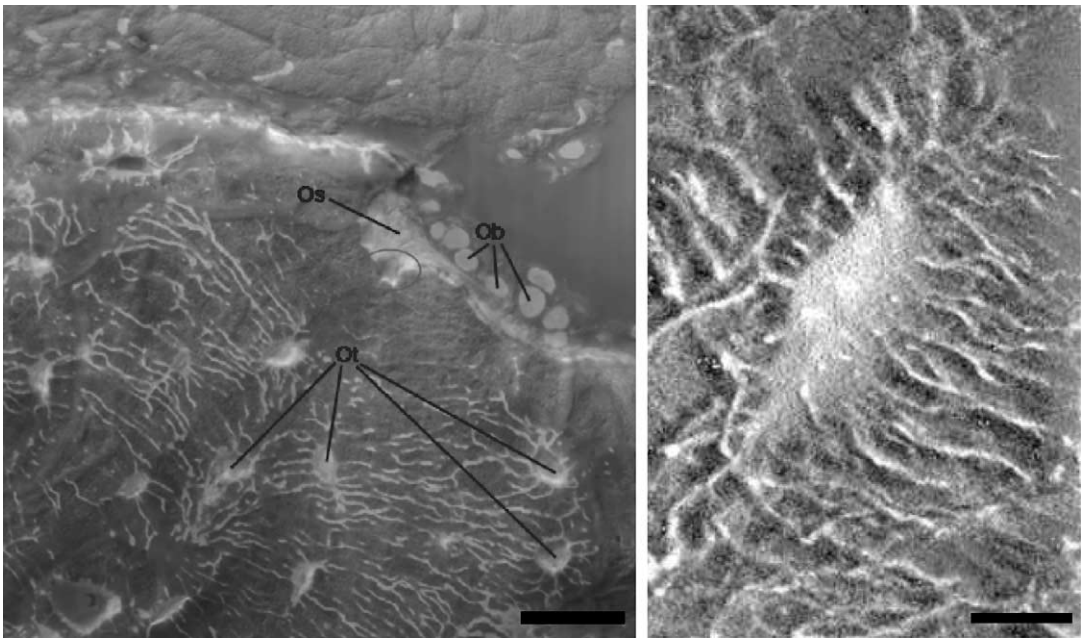

Fig. 1. (Left) Remodeling on the surface of a long bone. The bone was immobilized for a period and then remobilized prior to acquisition of the sample. Immobilization resulted in osteoclastic resorption and remobilization resulted in recruitment of osteoblasts (Ob) that fill in the osteoclastic resorption crater with osteoid (Os). During the infilling process, some osteoblasts are engulfed by osteoid, thereby becoming osteocytes (Ot, nascent osteocyte is circled). Scale bar $\sim 30\,\mu m$. (Right) Three-dimensional reconstruction of an osteocyte in situ in a thick bone section. Scale bar $\sim 5\,\mu m$. (After Knothe Tate, 2003.)

been dominated by histomorphometric studies of fixed specimens. Fixed specimens allow observation of morphology at one time point in the lifecycle of the cell, but the morphology and function of a given osteocyte depend upon the cell's prior differentiation history as well as its environment within the tissue (Figs. 1 and 2).

Osteocytes are derived from osteoprogenitors, a fraction of which differentiate into active osteoblasts (Ob, Fig. 1). Osteoblasts synthesize osteoid (Os), unmineralized bone matrix composed of collagen and other organic components. A fraction of the active osteoblasts become incorporated within the newly laid down matrix (Baud, 1968; Dudley & Spiro, 1961; Palumbo, 1986), and remain ensconced as osteocytes (Ot, Fig. 1) within spaces called lacunae. Nascent osteocytes (red oval, Fig. 1) maintain direct contact with the overlying bone lining cells and osteoblasts, as well as with previous generations of osteocytes through cell processes that are created before and during matrix synthesis (Fig. 1) (Baud, 1968; Dudley & Spiro, 1961; Palumbo, 1986). In mature bone, the osteocyte body and its processes are contained within spaces called lacunae and channels called canaliculi, respectively. Derived from the stellate shape of the osteocytes (Aarden, Burger, & Nijweide, 1994) and their interconnected cell processes, the lacunocanalicular system (LCS) is a conduit for metabolic traffic and exchange (Baud, 1968; Knothe Tate, 2003). The extended osteocytic network, comprised of cells interconnected by multiple cell processes and joined at gap junctions (Doty, 1981), forms a functional syncytium (Aarden et al., 1994; Knothe Tate, 2003). Thus, in addition to intercellular communication via the gap junctions (Doty, 1981), the cells making up the syncytial network remain in contact via their common environment that is defined by a contiguous fluid-saturated, proteoglycan network filled space (Knothe Tate, 2003).

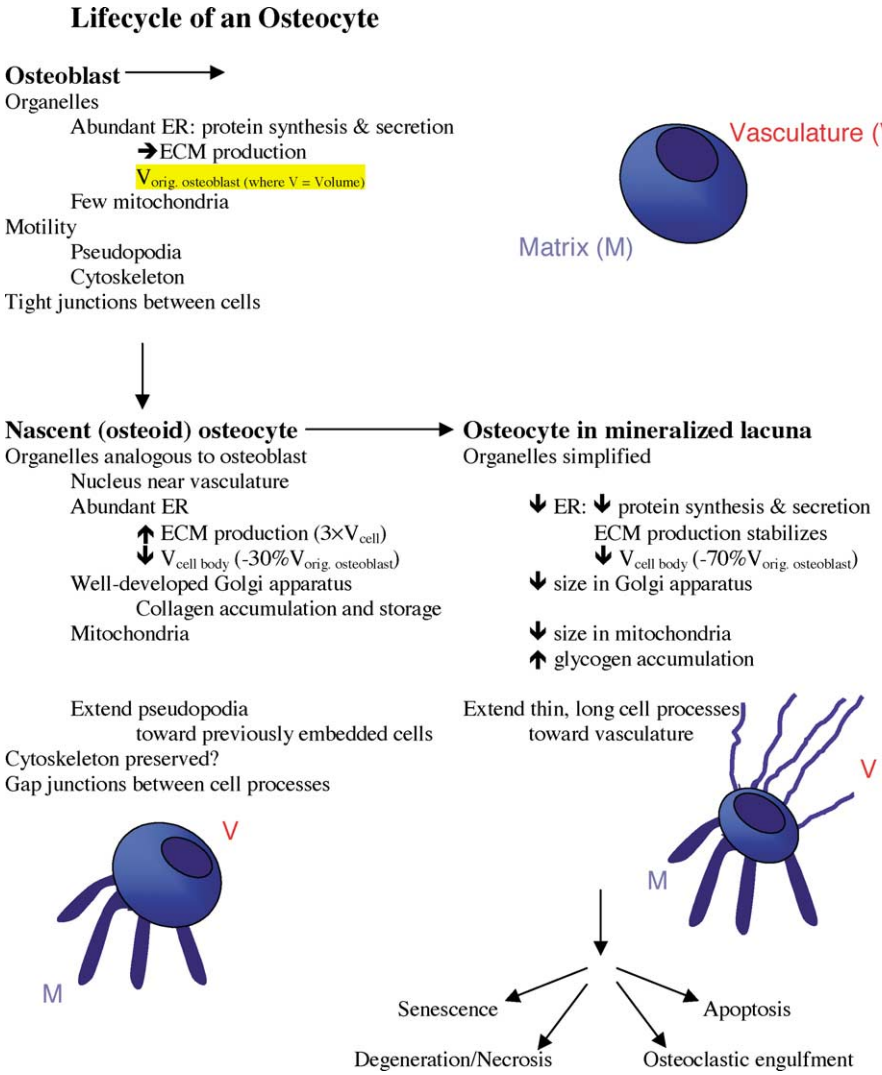

Fig. 2. The lifecycle of an osteocyte, from nascence to death.

3. Cell physiology

3.1. Lifecycle of an osteocyte

The stage of maturation of an osteocyte, from nascence until death, defines the morphology and function of the cell (Fig. 2). The transformation from motile osteoblast to entrapped osteocyte takes about 3 days during which time the cell produces a volume of extracellular matrix three times its own cellular volume (Palumbo, 1986). Cell polarity is maintained during the transformation from osteoblast to osteocyte, such that the nucleus remains in proximity to the vasculature, but a shift in cell volume distribution takes place, changing the rounded, active osteoblast to a more stellate or dendritic-shaped osteocyte. This results in a 30% volume reduction in the nascent osteocyte cell body and 70% volume reduction in the mature osteocyte cell body compared to the volume of the original osteoblast (Palumbo, 1986). The extent to which this reduction in cell body volume is accounted for by increased cell process volume is unknown but

is of interest since morphological changes associated with maturation of osteocytes could potentially affect their sensitivity to mechanical stimuli.

During transformation of the osteoblast to the nascent osteocyte, cell processes first radiate toward the mineralizing matrix; these processes are thick and pseudopod-like, and are thought to be involved in the extrusion of calcifying matrix vesicles (Palumbo, 1986). Once the mineralization front surrounds the cell, cell processes of a longer, thinner nature are observed on the vascular side of the cell. In the mature state, there are generally more cell processes oriented toward vascularity than toward the mineralization front (Palumbo, 1986). Osteocytes cultured in vitro exhibit cytoskeletal elements including microtubules, intermediate filaments and actin filaments in the osteocyte cell body and processes, whereby maintenance of the unique osteocyte shape is attributed to actin filaments (Tanaka-Kamioka, Kamioka, Ris, & Lim, 1998). While osteoblasts require a cytoskeleton for chemotaxis, entrapped preosteocytes use the same cytoskeletal machinery to extend pseudopodia and cell processes, thus maintaining orientation and connectivity with cells near the cement line and blood supply, respectively.

3.2. Osteocyte organelles

The cell machinery of a given osteocyte reflects not only its stage in the lifecycle but also its functional state in the tissue (Fig. 3). Osteocytes have an approximate average half-life of 25 years (Frost, 1963), although their life expectancy may be highly variable (Marotti, Canè, Palazzini, & Palumbo, 1990). In the nascent osteocyte, the structure of organelles is qualitatively similar to that of osteoblasts (Dudley & Spiro, 1961) although the size and numbers of organelles are diminished (Palumbo, 1986). A single nucleus is typically located toward the vascular side and has one or two nucleoli as well as a membrane replete with nuclear pores (Dudley & Spiro, 1961; Palumbo, 1986). Similar to their neighboring osteoblasts, nascent osteocytes contain abundant endoplasmic reticulum, many free ribosomes and mitochondria and a prominent Golgi apparatus adjacent to the nucleus (Palumbo, 1986). This machinery allows for the rapid production

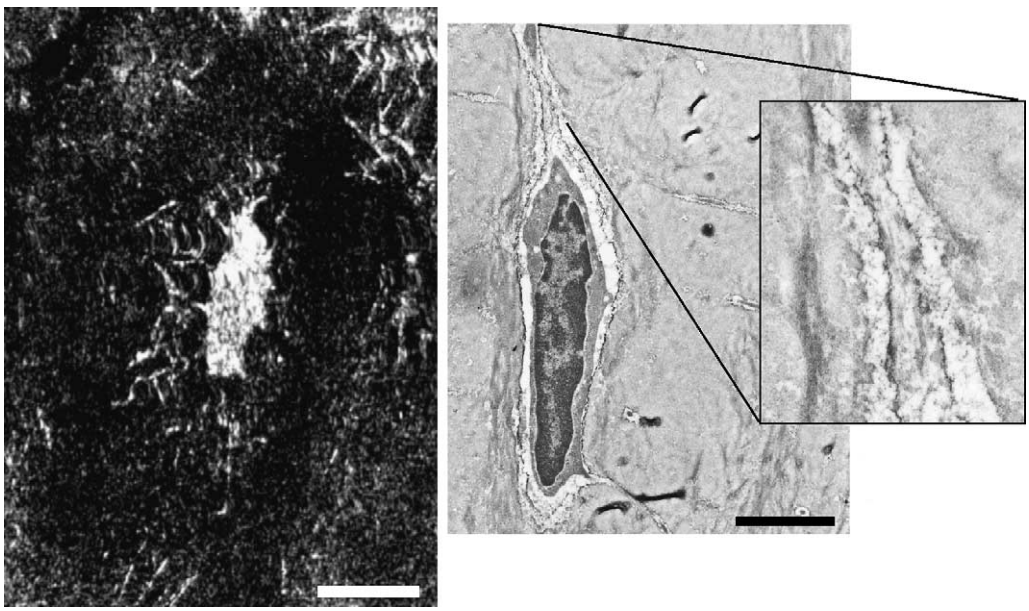

Fig. 3. (Left) In vivo and in situ image of an osteocyte from the sheep metacarpus, coronal plane, taken with a newly developed intraoperative microscopy system. Scale bar ∼ 8 μm. (Right) Transmission electron micrograph of a skeletally mature rat osteocyte, in cross-section, from the metacarpus, with inset showing osteocyte process encompassed by canalicular wall. Scale bar ∼8 μm.

of extracellular matrix composed of proteoglycans and collagen (Baud, 1968). The endoplasmic reticulum is less abundant in osteocytes surrounded by mineralized osteoid (Dudley & Spiro, 1961), indicative of their reduced production of extracellular matrix. In fact, with increasing distance from the mineralizing surface, or in mature tissues that are not undergoing modeling or remodeling, the appearance of granular endoplasmic reticulum, the Golgi apparatus and mitochondria decreases and appearance of glycogen accumulation increases (Doty, Robinson, & Schofield, 1976).

Osteocytes die as a consequence of senescence, degeneration/necrosis, apoptosis, and/or osteoclastic engulfment (Fig. 2), but the dynamic aspects of this life cycle are not yet defined. For example (Tomkinson, Reeve, Shaw, & Noble, 1997) reported that the percentage of dead osteocytes in bone increases with age from less than 1% at birth up to 75% beyond the eighth decade, and described a correlation between osteocyte death via apoptosis and conditions involving high bone turnover as well as proceeding estrogen withdrawal in human bone. Mullender, Van Der Meer, Huikes, and Lips (1996) on the other hand, showed no significant correlation between the percentage of empty lacunae and age in matched groups of control and osteoporosis patients. Recent studies indicate a relationship between diminishment in cell viability and the onset of osteoclastic resorption but the timing and signaling mechanisms for remodeling have yet to be elucidated. Finally, osteoclasts have been shown to engulf as well as to "liberate" entombed osteocytes through their resorptive activity, but the fate of the freed cell is unknown (Nijweide, Burger, & Klein-Nulend, 2002). There is little evidence that the osteocyte will revert back to osteoblastic state (Van der Plas et al., 1994).

3.3. Pericellular environment

The osteocyte's immediate pericellular environment has been described as a *Grenzscheide* or limiting membrane and consists of as yet undefined proteoglycans and extravascular fluid (Dudley & Spiro, 1961; Knothe Tate, 2003). This unmineralized area may aid in maintaining the integrity of osteocytes and their processes (Dudley & Spiro, 1961) by serving as a buffer zone to prevent mineralization into the lacunar space and thereby maintain patency of extracellular transport pathway. Increasing mineralization of the pericellular space results in a concomitant narrowing of the periosteocytic "buffer zone". Osteocytes show acid phosphatase activity and other lysosomal hydrolytic enzymes with the capacity to digest proteins and glycosaminoglycans; these are presumed to confer a means to modulate the pericellular buffer zone and to mobilize calcium in the pericellular matrix (Baud, 1968). An active role for this pericellular zone in enzymatically controlled depolymerization and repolymerization of proteoglycans has been suggested but not yet proven (Lipp, 1954). Furthermore, osteocytes in culture have the capacity to modulate their extracellular matrix environment through the production of matrix proteins including osteocalcin, osteonectin and osteopontin; not only would such modulation of the extracellular matrix be expected to result in changes to the biochemical milieu of the cell but it may also provide a strategy for adaptation of the mechanical environment at a cellular level (Aarden, Wassenaar, Alblas, & Nijweide, 1996).

The lacunocanalicular network provides a microcirculatory system for periosteocytic fluid that is distinct from the blood plasma and lymph fluid. This fluid not only defines the local biochemical environment of the cell but also serves as the coupling medium through which mechanical forces are translated into mechanobiological, biochemical, and electromechanical effects at a cellular level (see Klein-Nulend et al., 1995; Knothe Tate, 2003 for a review of these cellular effects). Metabolic activity of osteocytes influences the biochemical milieu of their surrounding fluid, although the exact biochemical composition and viscosity of this fluid is unknown, largely due to the practical difficulties of obtaining a sufficient sample for analysis. Important differences between bone extracellular fluid and plasma have been shown; in particular the concentration of K^+ is much higher in bone fluid than in plasma. Moreover, the amount of K^+ (mM/l) in bone extracellular fluid decreases with age and in states of metabolic deficiency (Canas, Terepka, & Neuman, 1969), providing a basis for differences in ionic content of periosteocytic fluid and the extravascular fluid bathing osteoprogenitor cells and osteoblasts on bone surfaces (Rasmussen & Bordier, 1974). Variations in water content have been documented as a function of species, age and underlying pathology of the specimen (Timmis and Wall, 1977).

4. Osteocyte functions

Bone is subjected to a dynamic environment in which functional adaptation is necessary for survival of the tissue and, ultimately, of the organism. Bone tissue health depends on the ability of bone cells to recognize and respond to mechanical and chemical stimuli, a process referred to as mechano-chemical transduction (see Knothe Tate, 2003 for a recent review). Remodeling activity, coordinated between osteocytes, osteoclasts, and osteoblasts (Fig. 1), provides a basis for adaptation. Osteocytes, the most abundant cells in bone, are actively involved in maintaining the bony matrix, and osteocyte death is eventually followed by matrix resorption (Junqueira, Carneiro, & Kelley, 1995). In addition, osteocytes are thought to be mechanosensors (Aarden et al., 1994; Burger & Klein-Nulend, 1999). Transmission of mechanical signals to the osteocyte cytoskeleton via cell surface receptors can occur directly through the solid matrix structure of the tissue as well as indirectly via fluid pressure and shear stresses imparted by fluid moving through the lacunocanalicular system due to load-induced fluid flow (see Knothe Tate, 2003 for recent review). Translation of mechanical signals at the cellular level may further involve triggering of integrin force receptors and/or changes in the conformation of membrane bound proteins that affect membrane fluidity and trafficking. In addition to these mechanical signals, chemical signals, modulated through diffusive, convective and active transport mechanisms, are transported intracellularly as well as through the extracellular fluid in which the cells are immersed. The lacunocanalicular system provides an ideal milieu for transfer of exogenous and endogenous signals via mechanical, electrical and chemical mechanisms. The cell signaling pathways leading to release of secondary messengers, transcription factors, and finally gene expression are not yet fully elucidated and are the subject of much current research.

5. Associated pathologies

The lifecycle and health of individual osteocytes influences the state of the cellular syncytium within bone tissue. The state of a given lacuna depends on the viability of the osteocyte contained within it. As osteocytes lose viability, their size and shape changes and their pyknotic remains may persist within the lacunae for some time. Thereafter, the remodeling cycle may be initiated to remove nonviable cell remains and surrounding tissue or a lacuna may remain empty, become mineralized and/or lose its patency (Currey, 1964; Frost, 1960). Hence, at a tissue level, the cellular syncytium and the common fluid space defined by this syncytium are interrelated and change, depending on the viability of individual cells and the state of the tissue (Knothe Tate, Tami, Bauer, & Knothe, 2002).

Histological observations suggest that the integrity and three-dimensional organization of the bone cell network change in disease states such as osteoporosis, osteoarthritis, and osteomalacia (Fig. 4). These changes point towards a cellular basis for many bone diseases. An understanding of these cellular changes may pave the way for new advances in prophylaxis and management of bone disease (Knothe Tate et al., 2002).

In particular, osteoporotic bone shows a disconnect between the ability to adapt through remodeling and the ability to bear the functional loads, thus predisposing itself to fracture and exacerbating the healing process. Most osteopenic bone is characterized by an increase in resorption spaces on bone surfaces and within the cortex (Fig. 1). The increase in osteoclastic resorption activity and decrease in osteoblastic infilling activity may be due to a decrease in osteoprogenitor cell populations or to altered feedback and signaling between bone cells due to a decrease in connectivity of the cell syncytium. The connectivity and orientation of the lacunocanalicular system are important predictors of cellular communication and mass transport. Hence, we expect them to have a profound effect on mechanotransduction in bone tissue, modulating the state of the tissue both in health and disease.

6. Outlook

The relative inaccessibility of osteocytes in the mineralized matrix has presented a challenge to their study and has stymied scientific advance. Today, a renewed interest in understanding the biology of these cells has provided impetus for the development of novel strategies to observe these cells in their natural environment. These will pave the way for the in situ and in

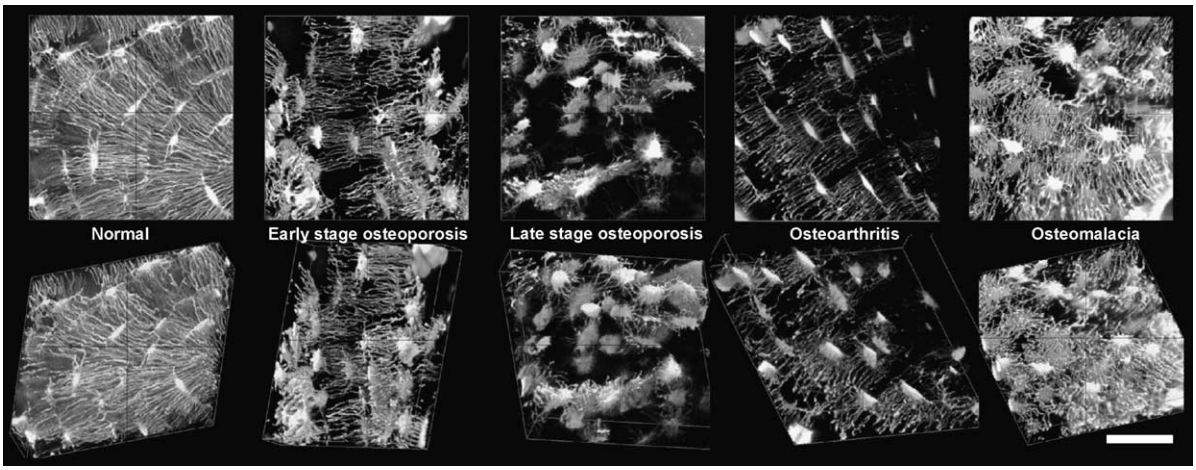

Fig. 4. Three-dimensional reconstructions of the osteocyte syncytium from human bone samples with specific bone diseases including osteoporosis, osteoarthritis and osteomalacia. Specimens were taken from tissue excised during hip replacement surgery and areas imaged were from tissue of the cortical sheath of the superior aspect of the femoral neck. The top row of images shows the volume of interest, looking in from the cross sectional plane. The bottom row of images shows the same volume at a slight angle, to aid in visualizing connectivity through the depth of the volume. In the undiseased human femur, osteocytes connectivity is high and processes are oriented in the direction of the blood supply (Normal). In contrast, the osteocytic network of osteoporotic bone shows a marked decrease in connectivity as well as orientation; processes exhibit higher tortuosity and appear slack (Early, late stage osteoporosis). Osteoarthritic bone exhibits a decrease in osteocyte viability and connectivity, even though the orientation of the processes remains intact (Osteoarthritis). Finally, osteocytes from osteomalacic bone appear viable with high connectivity, but cell processes are tortuous and the network is chaotic (Osteomalacia). Scale bar $\sim$ 20 μm (after Knothe Tate et al., 2002).

real-time observation of remodeling activity, unlocking the mystery of the timing and signaling mechanisms underlying bone remodeling in health and disease. Technology will broaden our perspective from an understanding of how bone tissue adapts to its dynamic environment to the degree to which pathological tissue is compromised in its ability to adapt. Perhaps that is the crux of the matter: just as cancer occurs when cells cannot stop proliferating, other bone diseases such as osteoporosis occur when tissues lose their ability to remodel due to loss of cell viability and subsequent loss in intercellular communication.

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