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PERSPECTIVES

The Role of Osteocytes in Functional Bone Adaptation

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Bone adaptation has attracted attention from a number of research disciplines. Mechanical engineers have tried to describe bone adaptation in terms of equations and computational models, clinicians and biologists have made observations of alterations in bone quality and quantity as a result of bed rest, paralysis and pharmaceutical treatments, and biochemists have investigated the signaling pathways and interactions between bone cells. The idea that the external shape and the internal structure of bone adapts to mechanical loading conditions dates back to 1638, when Galileo suggested that the shape of bones was related to mechanical loading. In 1892, Julius Wolff proposed a correlation between bone architecture and mechanical loading. He suggested that the trabecular architecture found in the proximal femur is orientated in the same direction as the stress trajectories that occur there. Roux, a contemporary of Wolff, suggested that bone adaptation was a self-regulating mechanism by which bone attempts to obtain maximum strength with minimum weight (1). By changing the shape of a bone and organising its internal structure, the amount of tissue required for bones to perform their function can be minimized.

Bone adaptation is a dynamic process that involves both modeling and remodeling. Modeling is a process by which bone changes in length and diameter during early development towards a mature skeleton. Unlike modeling, which involves either resorption or formation, bone remodeling follows an activation, resorption, and formation sequence (frost). Remodeling of bone requires a complex arrangement and interaction of cells, collectively called basic multicellular units (BMUs). Both processes are tightly controlled by a number of cell types. The four main types of cells that can be found in bone are bone lining cells, osteoblasts, osteocytes and osteoclasts. Bone lining cells, osteoblasts and osteoclasts are located on the bone surfaces, whereas the osteocytes are in the bone matrix. Osteoblasts and osteoclasts are the bone forming and bone resorbing cells, respectively. Osteocytes and lining cells are derived from osteoblasts that have stopped producing bone matrix (2). Palumbo *et al.* (2) proposed that pre-osteoblasts mature into osteoblasts, some of which are committed to differentiate further. These committed osteoblasts start to lose their activity and are buried inside the bone matrix by adjacent osteoblasts. Consequently, pre-osteocytes start the formation of cellular processes in the mineral-facing side and become osteoid osteocytes. The mature osteocyte radiates processes in all directions and is located within lacunae inside the hard mineralized matrix.

Experimental Studies

Mechanically-induced bone adaptation has been proposed to be a result of strain magnitude (3), stress (4), cycle number, strain history, strain rate, strain energy density (5) and frequency of specific stimuli. Other researchers have developed theoretical models based on the idea that adaptation is controlled by the level of fatigue damage, i.e., the number and length of cracks (6;7). Martin (7), for example, used the amount of damage (mean crack length times crack density) as the stimulus for bone remodeling. In his model, the activation frequency of BMUs was directly related to the amount of damage present in a local

area. As a consequence of damage formation, BMUs are activated, thereby increasing the porosity, resulting in increased strain. Subsequently, this promotes damage accumulation, which is not considered to be dangerous, while the load remains below a critical level. The increase in damage removal then overtakes the rising damage formation rate, resulting in a new state of equilibrium. Conversely, when the effective strain levels are below a critical value, BMUs are activated, causing resorption of bone. This idea is attractive because the level of damage provides a direct measure of the potential danger of failure. If the level of damage, and particularly its rate of increase, is greater than can be repaired, then failure will occur unless adaptation is initiated to reduce the stress level. An advantage of this approach is that it automatically accounts for the dynamic loading history as the driving force for the remodeling process. However, while the concept of damage-stimulated remodeling and adaptation is appealing, it suffers from a number of problems, in particular, the lack of knowledge of how bone detects damage and 'decides' to initiate repair or adaptation.

Researchers have come to believe that the altered loading environment causes passive and active cellular responses mediated by mechanotransduction, which is thought to cause altered gene and protein expression (biochemical signaling) (8). Osteocytes, with their fine cell-processes radiating from the cell, are believed to fulfill this function (8;9). It has been hypothesised that transmission of mechanical signals to the osteocyte skeleton via cell surface receptors (10) can occur directly through the solid matrix of the tissue due to load-induced fluid flow (8;11-13), as well as indirectly via fluid pressure and shear stresses (9;14) through the lacuno-canalicular system. This network allows communication and transport of organic and inorganic matter between cells deep in the tissue and those located in the vicinity of vascular canals and bone surfaces. All this requires bone cells to be sensitive to mechanical and chemical stimuli.

Osteocytes

Osteocytes form an interconnected network through their dendritic processes, allowing communication between individual osteocytes and the bone surface lining cells. Although the functional role of the osteocyte remains partly unknown, a key role in the regulation of remodeling of the skeletal architecture in response to mechanical load has been suggested. The mechanism(s) by which osteocytes orchestrate and control bone formation and resorption plays a pivotal role in current bone research. Marotti *et al.* (15) suggested that an inhibitory signal is generated by the osteocytes that is passed through their cell processes to osteoblasts for recruitment to enable bone formation. Martin (16) extended this concept by developing a hypothetical model for the down-regulation of remodeling by osteocytes. He proposed that the strength of the inhibitory signal perceived by each osteoblast is proportional to the number of osteocytes connected to the lining cells located on quiescent bone surfaces, which is inversely proportional to the distance involved, to prevent initiation of a remodeling sequence. Regions of bone exhibiting high rates of remodeling were shown to correlate with relatively low numbers of viable osteocytes, because low osteocyte density would reduce the proposed functional brake to remodeling (17). This is interesting since it has been reported that osteocytes in woven bone appeared at an early stage of bone repair and developed a few canaliculi, typically short and irregularly distributed in the osteoid matrix. Osteocytes in lamellar bone formed many canaliculi that are long and regularly distributed in mature bone matrix. This might indicate that woven bone osteocytes may be necessary for induction of the lamellar bone osteocytes followed by active appositional growth of lamellar bone at an early stage of bone repair, based on the pattern of development of bone canaliculi by the osteocytes (18). Other studies have confirmed this. It has been estimated that the osteocyte population in woven bone is approximately four to eight times as large as that in lamellar bone (19), which might indicate that woven bone, with increased lacunar density, differs from lamellar bone and undergoes remodeling at

an accelerated rate (20). Given the potentially important role of osteocytes, various studies have been performed to gain more insight into the morphology of these cells using immunofluorescence microscopy (21-23). The cell body varies in size, ranging from approximately 5-20 μm in diameter (22), with a volume of 257 μm^3 occupying approximately 724 μm^2 (23). Osteocytes contain between 40 and 60 cell processes per cell, with a cell-to-cell distance between 20 and 30 μm (23), and a diameter varying from 50 to 410 nm (24). However, there are still questions that remain unanswered. For example, African Americans have higher BMD-indexes (25), lower osteon densities (26), higher osteocyte densities (27) and are less prone to stress fractures (28), compared to white Americans. Do these various parameters contribute to a reduction in stress fracture and therefore the accumulation and initiation of microdamage?

Detection of Microdamage by Osteocytes

It has been known for over 40 years that bone *in vivo* contains small cracks, typically 100 μm long (29-31). These cracks are the visual manifestation of fatigue damage, caused by the cycles of stress that occur during daily activities. A number of researchers have noted that the vast majority of the cracks can be found in older bone located between the osteons. The percentage of cracks found in interstitial bone by various researchers were 87%, 62.4% and 85%, respectively (32;33). Recently, Qui *et al*. (34) found that regions, such as interstitial bone, with osteocyte densities less than 728/mm^2 are 3.8 times more likely to contain microdamage than regions with higher osteocyte densities. The question remains whether microdamage can trigger bone adaptation and what functions the osteocytes serve in this process. Using the isolated ulna loading model, intracortical bone remodeling in rats was shown by Bentolila *et al.* (35) to be triggered by fatigue loading. In this experiment, 14 out of 16 rats showed microcracks in the bone cortex. After 10 days of fatigue loading, resorption cavities were observed. However, two rats had no microdamage following fatigue loading. In these two specimens, no resorption cavities were observed, providing more evidence that microdamage and bone remodeling are linked. The evidence that microdamage can trigger bone modeling is, to date, only circumstantial. For example, Hsieh *et al.* (36) and Lee *et al*. (37) also used the isolated ulna loading model and reported bone deposition. However, they found no evidence of microdamage-induced bone formation. This result was rather surprising given the loading amplitude and frequency used for the experiments. However, what these studies did show was that it is irrelevant how many load cycles to which bones are subjected because the stimulus caused by the induced strain saturates. In these studies, there was an asymptotic approach to saturation as the duration of loading was increased from 36 to 720 cycles per day. Increasing the duration of a loading bout therefore resulted in diminishing returns in bone formation, again suggesting that cells may become non-responsive to repeated mechanical stimuli (38). Even if these studies showed that the effective altered strain would diminish, microdamage might indirectly cause a sustained local stimulus. Microdamage causes a local stress concentrator around its perimeter. If strain levels do not decrease, cracks will continue to propagate and therefore the local strains, caused by these cracks, will continue to increase (39). It has been suggested that increased strain levels cause osteocytic apoptosis due to the presence of microcracks that affect osteocyte homeostasis (32;40;41). Recent experimental work that has tried to investigate this phenomenon has indicated that osteocyte apoptosis increases osteoclastic activity (42).

There still remain several questions regarding the interaction between osteocytes and the presence of microdamage. If bone is the activation signal to trigger BMU activity, how can bone cells detect the presence of cracks? It is highly plausible that such a system would be in place. If all microcracks were removed from the matrix, the mechanical integrity of bone might be compromised since tunneling BMUs would weaken the structure. On the other hand, if microdamage remained undetected, various microcracks might propagate to form macrocracks leading to

fracture. But how do the osteocytes decide whether a crack is dangerous enough to require repair? If this principle were extended, and the rate of microdamage accumulation were so high that the remodeling process could not keep up, then the only way to reduce propagation would be to deposit new bone at surfaces in order to reduce strain levels. But how do the osteocytes decide whether the level of damage is high enough to require surface adaptation? A possible answer to this question might lie in damage to the cell processes. Recently, a theoretical model was developed based on this principle.

A typical microcrack is elliptical in shape, with the minor axis having a length of 100 μm and the major axis measuring lengths up to 700 μm, orientated at approximately 20 degrees to the longitudinal axis of the bone (43). Given that human cortical bone contains between 13,900 to 19,400 osteocytes per mm^3 (44) with 40-60 cell processes each, it is likely that the microcrack crosses this network. Since microcracks tend to propagate in a direction similar to the local lamellar orientation, it would mean that cracks subjected to tensile loading open up and produce shear displacement. Cracks subjected to compressive loading would result in compression of the fracture surfaces and additional shear displacement, resulting in the rupture of cell processes. Using linear elastic fracture mechanics and various osteocyte densities, it was predicted that small microcracks (less than 30 μm) would not produce enough shear displacement to rupture cell processes, independent of the stress level to which these cracks were subjected. Microcracks with a typical length of 100 μm showed ruptured cell processes at stress levels exceeding 28 MPa. If the stress levels were increased for cracks of this length, several hundred cell processes would rupture. Extremely large cracks (greater than 300 μm) tend to rupture several thousand cell processes even at extremely low stress levels (39). Given that few crack lengths in excess of 100 μm have been reported in the literature, this might indicate that cell process rupture plays an important role in monitoring local microdamage accumulation. Experimental evidence, in which propagating cracks were monitored under a UV-epiflourescence microscope and additional staining of the cytoskeleton, showed that the majority of cell processes rupture between the two crack faces. Only near the crack tip, a region where crack face displacements are negligible, did cell processes remain intact (45). The question remains how osteocytes respond to damaged cell processes.

This process can only be understood if the cell-to-cell interaction, and therefore the cellular network in bone, is understood from a biochemical point of view. A novel approach to investigate the effect of microdamage to the cellular network was published recently by Heino *et al.* (46). In their study, damage was introduced to a three-dimensional osteocyte cell culture (using the MLO-Y4 cell line) with a 21-gauge needle. It was found that the introduction of microdamage had no significant effect on the number of dead cells compared to the control. However, the bone marrow cells, which were seeded on top of the gel, were TRACP positive in a region close to the damaged site. Furthermore, it was shown, using an ELISA, that the gel embedded osteocytes secreted significant amounts of both M-CSF and RANKL. The secretion of these osteoclastogenic factors was further enhanced by introducing mechanical stretching of the gel cell culture. These results indicated that osteocytes are affected by microdamage and that they play a significant role in orchestrating the activity of osteoclasts and osteoblasts. Previous work by the same group (47-50) showed that signaling pathways and the secretion of various proteins play an important role in the regulation and maintenance of the mechanical integrity of bone.

All of this raises the question: are the differences in remodeling rate in woven bone and lamellar bone related to osteocyte density? Furthermore, is the decrease in osteocyte density with age a key factor in repair and prevention of osteoporotic fractures? If microdamage is detected through ruptured cell processes and therefore the secretion of chemicals to initiate repair, could it be that the sensing mechanism in older bone is compromised,

resulting in an underestimation of microcrack length and potential danger to the mechanical integrity of the structure itself?

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