韓國精密工學會 2002年度 秋季學術大會論文集 pp.1055~1058 KSPE 02F098

BONES HAVE EARS

Stephen C. Cowin

The New York Center for Biomedical Engineering and
The Departments of Biomedical and Mechanical Engineering
The School of Engineering of The City College and
The Graduate School of The City University of New York
New York, NY 10031, U. S. A.

The movement of bone fluid from the region of the bone vasculature through the canaliculi and the lacunae of the surrounding mineralized tissue accomplishes three important tasks. First, it transports nutrients to the osteocytes in the lacunae buried in the mineralized matrix. Second, it carries away the cell waste. Third, the bone fluid exerts a force on the cell process, a force that is large enough for the cell to sense. This is probably the basic mechanotransduction mechanism in bone, the way in which bone senses the mechanical load to which it is subjected. The mechanisms of bone fluid flow are described below with particular emphasis on mechanotransduction. Also described is the cell to cell communication by which higher frequency signals might be transferred, a potential mechanism in bone by which the small whole tissue strain is amplified so the bone cells can respond to it. One of the conclusions is that higher frequency low amplitude strains can maintain bone as effectively as low frequency high amplitude strains. This mechanism has many similarities with the mechanotransduction of acoustical signals in the ear. These conclusion leads to a paradigm shift in how to treat osteoporosis and how to cope with microgravity.

The syncytium of osteocytes, bone-lining cells, and osteoblasts

The cells that lie directly on the bony surfaces are bone-lining cells, osteoblasts, and osteoclasts. Osteoblasts and osteoclasts are the effector cells of bone formation and bone resorption, respectively. The bone cells that are buried in the extracellular bone matrix are the osteocytes. Each osteocyte, enclosed within its mineralized lacuna, has many (perhaps as many as 60) cytoplasmic processes. These processes are approximately 15 mm long and are arrayed three-dimensionally in a manner that permits them to interconnect with similar processes of up to 12 neighboring cells. These processes lie within mineralized bone matrix channels called canaliculi. The small space between the cell process plasma membrane and the canalicular wall is filled with bone fluid and macromolecular complexes that are thought to form a glycocalyx on the cell process surface. The bone-lining cells, osteoblasts, and osteocytes (i.e. all bone cells except osteoclasts) are extensively interconnected by the cell process of the osteocytes forming a syncytium (Cowin et al. 1991; Cowin and Moss, 2000).

The touching cell processes of two neighboring bone cells contain gap junctions. A *gap junction* allows ions and compounds of low molecular weight to pass between the two cells without passing into the extracellular space. Gap junctions connect superficial osteocytes to periosteal and endosteal bone-lining cells and osteoblasts. All bone surface cells are similarly interconnected laterally on a bony surface. Gap junctions are found where the plasma membranes of a pair of markedly overlapping canalicular processes meet. In compact bone, canaliculi cross the cement lines that form the outer boundary of osteons. Thus extensive communication exists between osteons and interstitial regions. Live bone cells allow the active intercellular transmission of ions and small molecules through gap junctions. In a physical sense, the bone cell syncytium represents the hard wiring (Cowin et al. 1991; Cowin and Moss, 2000) of bone tissue.

The osteocyte as the sensor of mechanical loading

There is only circumstantial evidence that the osteocyte is the primary mechanosensory cell in bone tissue. A list of that evidence is as follows:

a. The placement and distribution of osteocytes in the three-dimensional labyrinthine syncytium is architecturally well suited to sense deformation of the mineralized tissue encasing them. Further, the syncytium provides an intracellular as well as an extracellular route for rapid passage of ions and signal

molecules. A contrary argument is that, if the bone cell syncytium does not sense mechanical loading, what does it do?

b. The only other candidates for the role of the primary mechanosensory cell in bone tissue are the osteoblasts, the bone lining cells and the osteoclasts. The osteoclasts may be eliminated directly because they are present in the bone tissue only when they are accomplishing their resorption function. Bone-lining cells should probably be considered as surface osteocytes because they likely represent the last group of osteoblasts on a (re)modeling bone surface, osteoblasts that ceased activity and flattened out because the bone surface was complete. Thus the only other serious candidate is the osteoblast. It should be anticipated that, if the osteocyte has some mechanosensory capacity, then so should the osteoblast because the osteoblast is the progenitor of the osteocyte. However the location of the osteoblasts on bone surfaces means that they must generally sense strain through their supporting substratum and, since the strain in the bone is small (0.2%) this requires very great cell sensitivity (Cowin et al. 1991). Furthermore, there is generally a layer of osteoid between the surface cell and the mineralized matrix, compromising the contact. The osteocyte, on the other hand is in a situation to directly sense the bone strain fluid movement as we describe in the following section.

c. The osteocyte has been shown to be extremely sensitive to fluid shearing stress, but not to cyclic hydrostatic compressive stress. Chicken osteocytes were shown to be the most stress sensitive cells of bone, capable of rapidly transducing mechanical stress into chemical messengers such as prostaglandins and nitric oxide. A number of osteoblastic and osteocytic cells have been shown to have the same sensitivity (You et al., 2001).

Mechanical stimulation of the osteocyte

The *stimulus* for bone remodeling is defined as that particular aspect of the bone's stress or strain history that is employed by the bone to sense its mechanical load environment and to signal for the deposition, maintenance or resorption of bone tissue. The case for *strain rate* as a remodeling stimulus has been building over the last quarter century. Animal studies have suggested the importance of strain rate, as opposed to strain, as a remodeling stimulus. Studies (Weinbaum et al., 1994; Cowin et al., 1995; Wang et al., 1999; You et al., 2001) directed at the understanding of the cellular mechanism for bone remodeling have suggested that the prime mover is the bone strain rate driven motion of the bone fluid. The motion of the bone fluid over the osteocyte is sensed by the osteocyte. It was proposed that the osteocytes are stimulated by relatively small fluid shear stresses acting on the membranes of their osteocytic processes. A hierarchical model of bone tissue structure, which related the cyclic mechanical loading applied to the whole bone to the fluid shear stress at the surface of the osteocytic cell process, was presented (Weinbaum et al., 1994). In this model the sensitivity of strain detection is a function of frequency; in the physiological frequency range (1-20 Hz), associated with either locomotion (1-2 Hz) or the maintenance of posture (15-30 Hz), the fluid shear stress is nearly proportional to the product of frequency and strain. Thus if bone cells respond to strains on the order of 0.1% at frequencies of one or two Hz, they will also respond to strains on the order of 0.01% at frequencies of 20 Hz. The fluid shear stresses will also strain the macromolecular mechanical connections between the cell and the extracellular bone matrix mentioned in the section above; thus fluid shear stress is also potentially capable of transmitting information from the strained matrix to the bone cell membrane. Extracellular matrix macromolecules connect via integrins in the cell membrane to the cytoskeleton.

Skeletal muscle contraction is a typical bone-loading event and has been implicated as a stimulus of bone cell activity. Frequency is one of the critical parameters of the muscle stimulus and it serves to differentiate this stimulus from the direct mechanical loads of ambulation, which occur at a frequency of one to two Hz. The frequency of contracting muscle in tetanus is from 15 Hz to a maximum of 50 - 60 Hz in mammalian muscle. It has been observed that these higher order frequencies, significantly related to bone adaptational responses, are "...present within the muscle contraction strain energy spectra regardless of animal or activity". The close similarity of muscle stimulus frequencies to bone tissue response frequencies is discussed below.

Response of the osteocyte to fluid flow and pressure

It has been shown that osteocytes, but not periosteal fibroblasts, are extremely sensitive to fluid flow, resulting in increased prostaglandin as well as nitric oxide production. Three different cell populations, namely osteocytes, osteoblasts, and periosteal fibroblasts, were subjected to two stress regimes,

pulsatile fluid flow and intermittent hydrostatic compression. Intermittent hydrostatic compression was applied at a frequency of 0.3 Hz with an amplitude of 13 kPa. The pulsatile fluid flow applied had a mean shear stress of 0.5 Pa and cyclic variations with an amplitude of 0.02 Pa at a frequency of 5 Hz. The maximal hydrostatic pressure rate was 130 kPa/sec and the maximal fluid shear stress rate was 12 Pa/sec. Under both stress regimes, osteocytes appeared more sensitive than osteoblasts, and osteoblasts more sensitive than periosteal fibroblasts. However, despite the large difference in peak stress and peak stress rate, pulsatile fluid flow was more effective than intermittent hydrostatic compression. Osteocytes, but not the other cell types, responded to one-hour pulsatile fluid flow treatment with a sustained prostaglandin E_2 up regulation lasting at least one hour after pulsatile fluid flow was terminated. By comparison, intermittent hydrostatic compression needed six hours' treatment before a response was detected. These results suggested that osteocytes are more sensitive to mechanical stress than osteoblasts, which are again more sensitive than periosteal fibroblasts. Furthermore, osteocytes appeared particularly sensitive to fluid shear stress, more so than to hydrostatic stress. Recently it has been shown that substrate deformation probably plays less of a role in bone cell mechanotransduction than fluid flow (You, et al., 2001). These conclusions are in agreement with the theory developed in (Weinbaum et al., 1994; Cowin et al., 1995) suggesting that osteocytes are the main mechanosensory cells of bone, and that they detect mechanical loading events by the canalicular flow of interstitial fluid that results from that loading event. Biot's porous media theory was employed to relate loads applied to a whole bone to the flow of canalicular interstitial fluid past the osteocytic processes in (Weinbaum et al., 1994) and (Cowin et al., 1995). Their calculations predict fluid induced shear stresses of 0.8-3 Pa, as a result of peak physiological loading regimes. The findings that bone cells in vitro actually respond to fluid shear stress of 0.2-6 Pa lend experimental support to this theory.

Osteocytes also rapidly release nitric oxide in response to stress and this nitric oxide response seems to be required for the stress-related prostaglandin release. Therefore, the behavior of osteocytes compares to that of endothelial cells that regulate the flow of blood through the vascular system, and also respond to fluid flow of 0.5 Pa with increased prostaglandin and nitric oxide production. The response of endothelial cells to shear stress is likely related to their role in mediating an adaptive remodeling of the vasculature, so as to maintain constant endothelial fluid shear stress throughout the arterial site of the circulation.

Strain amplification

There is an interesting paradox in our current understanding of bone physiology. The paradox is that the strains applied to whole bone (*i.e.*, tissue level strains) are much smaller (0.04% to 0.3%) than the strains (1% to 10%) that are necessary to cause bone signaling in deformed cell cultures (Fritton et al. 2000; You et al. 2001). There then must exist a mechanism by which the strain is amplified in the bone tissue so that sufficient magnitude strains occur at the cellular level. The answer to this paradox will be an important link in the mechanosensory system in bone and in relating in vitro cell studies to in vivo cellular response. In You et al. 2001 a mechanism whereby the flow of bone fluid through the glycocalyx surrounding an osteocytic cell process will lead to strains of the appropriate magnitude in the cell process membrane is described and modeled. In this speculative strain amplification mechanism, amplification by a factor of at least several hundred is possible. Whether strain amplification occurs or not depends upon the values of certain physical and biological parameters. These parameters include the elastic constants of the cell membrane, the elastic constants of the fibers that constitute the glycocalyx, the pre-tension in the fibers and cell membrane, the permeability of bone fluid through the glycocalyx, the physical dimensions of the cell process, the canaliculus, the fibers and fiber spacing of the glycocalyx. The model of You et al. 2001 assumed that: 1) the process is 100 nm in diameter; 2) the canaliculus is 200 nm in diameter; 3) there is a fiber matrix (the glycocalyx) in the annular space between the cell process membrane and the canalicular wall which connects the process membrane to the canalicular wall; 4) there are transverse elements in the pericellular matrix extending from the process membrane to the canalicular wall; 5) there are actin bundles inside the osteocyte process; 6) the distance between the actin filaments in the actin bundle is 25nm.

Osteocyte to bone surface cell communication

From a communications viewpoint the syncytium is a multiply noded (each osteocyte is a node) and multiply connected network. Each osteocytic process is a connection between (at least) two osteocytes,

and each osteocyte is multiply connected to a number of osteocytes that are near neighbors. In order to transmit a signal over the syncytium one osteocyte must be able to signal a neighboring osteocyte who will then pass the signal on until it reaches bone cells on the bone surface. There are a variety of chemical and electrical cell-to-cell communication methods. The passage of chemical signals, such as Ca^{2+}, from cell to cell appears to occur at a rate that would be too slow to respond to the approximately 30 Hz signal associated with muscle firing. We focus here on electrical cell-to-cell communication. A cable model was formulated in for cell-to-cell communication in an osteon. The spatial distribution of intracellular electric potential and current from the cement line to the lumen of an osteon was estimated as the frequency of the loading and conductance of the gap junction were altered. In this model the intracellular potential and current are driven by the mechanically induced strain-generated streaming potentials (SGP) produced by the cyclic mechanical loading of bone. The cable model predicts that the connected osteocytic processes function as a high-pass, low-pass filter. The generation of the streaming potentials is a high pass filter because the SPG generation rises from zero at zero frequency to a plateau with respect to frequency. The decay of the signal along the connected osteocytic processes functions as a low-pass filter because higher frequencies are not propagated. The theory also predicts that the pore pressure relaxation time for the draining of the bone fluid into the osteonal canal is of the same order as the characteristic diffusion time for the spread of current along the membrane of the osteocytic processes. This coincidence of characteristic times produced a spectral resonance in the cable at 30 Hz. Thus there is a large amplification of the intracellular potential and current in the surface bone cells which could serve as the initiating signal for a remodeling response. This voltage amplification might also explain why live bone appears to be selectively responsive to the mechanical loading in a specific frequency range (15-60 Hz), as has been experimentally demonstrated for several species.

The primacy of electrical signals is suggested here, since while bone cell transduction may also use small biochemical molecules that can pass through gap junctions, the time-course of mechanosensory processes is believed to be too rapid for the involvement of secondary messengers. As we noted above, the passage of chemical signals, such as Ca^{2+}, from cell to cell appears to occur at a rate that would be too slow to respond to the approximately 20 -30 Hz signal associated with muscle firing.

The mechanotransduction mechanism described above for living bone tissue has many similarities with the mechanotransduction system for acoustical signals in the ear.

References

Cowin, S.C., Moss-Salentijn, L., and Moss, M.L. (1991)Candidates for the mechanosensory system in bone. *J. Biomech. Engin. 113:191-197.*

Cowin, S.C., Weinbaum, S., and Zeng, Y. (1995) A case for bone canaliculi as the anatomical site of strain generated potentials, *J. Biomechanics,* 28:1281-1296.

Cowin, S. C. and Moss, M.L. (2000) Mechanosensory mechanisms in bone, in *Textbook of Tissue Engineering*, (2^{nd} edition) Lanza, R., Langer, R., and Chick, W. eds., Academic Press, San Diego, pp 723-738.

Fritton, P.S., Kenneth, J.M. and Rubin, C.T. (2000) Quantifying the Strain History of Bone: Spatial Uniformity and Self-similarity of Low Magnitude Strains. *J. Biomechanics,* 33:317-325.

Weinbaum, S., Cowin, S.C., and Zeng, Y. (1994) Excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. *J. Biomechanics,* 27:339-360.

Wang, L., Fritton, S. P., Cowin, S.C., and Weinbaum, S. (1999) Fluid pressure relaxation mechanisms in osteonal bone specimens: modeling of an oscillatory bending experiment, *J. Biomechanics*, 32:663-672.

You, L., Cowin, S.C., Schaffler, M. and Weinbaum, S. A model for strain amplification in the actin cytoskeleton of osteocytes due to fluid drag on pericellular matrix, *J. Biomechanics*, 34:1375-1386, 2001.