Bones have ears

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The mechanosensory mechanisms in bone include: (i) the cell system that is stimulated by external mechanical loading applied to the bone; (ii) the system that transduces that mechanical loading to a communicable signal; and (iii) the systems that transmit that signal to the effector cells for the maintenance of bone homeostasis and for strain adaptation of the bone structure. The effector cells are the osteoblasts and the osteoclasts. These systems and the mechanisms that they employ have not yet been unambiguously identified. The candidate systems are reviewed here. In particular a summary is given of the current theoretical and experimental evidence suggesting that osteocytes are the principal mechanosensory cells of bone, that they are activated by the effects of fluid flowing through the osteocyte canaliculi, and that the electrically coupled three-dimensional network of osteocytes and lining cells is a communications system for the control of bone homeostasis and structural strain adaptation. The similarities of the mechanotransduction system in bone with the mechanotransduction system used by the cells of the hearing system will be described. Both cell systems sense mechanical vibrations in a fluid domain.

Key words: *mechanosensation, bone, hearing, cell systems.*

1. Introduction

It has long been known that living adult mammalian bone tissue adapts its material properties, and that whole bones adapt their shape, in response to altered mechanical loading [1, 2, 3, 4, 5, 6, 7, 8]. Progress is being made in understanding the cellular mechanisms that accomplish the absorption and deposition of bone tissue. The physiological mechanism by which the mechanical loading applied to bone is sensed by the tissue, and the mechanism by which the sensed signal is transmitted to the cells which accomplish

the surface deposition, removal and maintenance, have not been identfied. The purpose of this contribution is to review some of the background research on these mechanosensory mechanisms and to outline candidates for the mechanosensory system. See [9, 10] for earlier reviews of similar literature.

Mechanoreception is the term used to describe the process that transmits the informational content of an extracellular mechanical stimulus to a receptor cell. *Mechanotransduction* is the term used to describe the process that transforms the mechanical stimulus' content into an *intra*-cellular signal. The term *mechanosensory* is employed to mean both mechanoreception and mechanotransduction. Additional processes of *inter*-cellular transmission of transduced signals are required at tissue, organ and organismal structural levels. The mechanosensing process(es) of a cell enables it to sense the presence of, and to respond to, extrinsic physical loadings. This property is widespread in uni- and multicellular animals [11, 12, 13, 14, 15, 16], plants [17, 18] and bacteria [9]. Tissue sensibility is a property of a connected set of cells and it is accomplished by the intracellular processes of mechanoreception and mechanotransduction.

2. The connected cellular network (CCN)

The bone cells that lie on all bony surfaces are osteoblasts, either active or inactive. Inactive osteoblasts are called bone-lining cells; they have the potential of becoming active osteoblasts (Fig. 1). 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 80) cytoplasmic processes (Figs. 1 and 2). These processes are approximately 15 μm long and are arrayed three-dimensionally in a manner that permits them to interconnect with similar processes of up to as many as 12 neighboring cells. These processes lie within mineralized bone matrix channels called canaliculi (Figs. 2 and 3). The small space between the cell process plasma membrane and the canalicular wall is filled with bone fluid and macromolecular complexes of unknown composition. All bone cells except osteoclasts are extensively interconnected by the cell process of the osteocytes forming a *connected cellular network* (CCN) [20, 21, 22]. The interconnectivity of the CCN is graphically illustrated by Fig. 4 which is a scanning electron micrograph showing the replicas of lacunae and canaliculi in situ in mandibular bone from a young subject aged 22 years. The inset of Fig. 4 shows enlarged lacunae identified by a rectangle.

The touching cell processes of two neighboring bone cells contain gap junctions [23, 24, 25, 26, 27, 28, 29, 30]. A *gap junction* is a channel connecting two cells. The location of the gap junction is indicated by the arrowheads
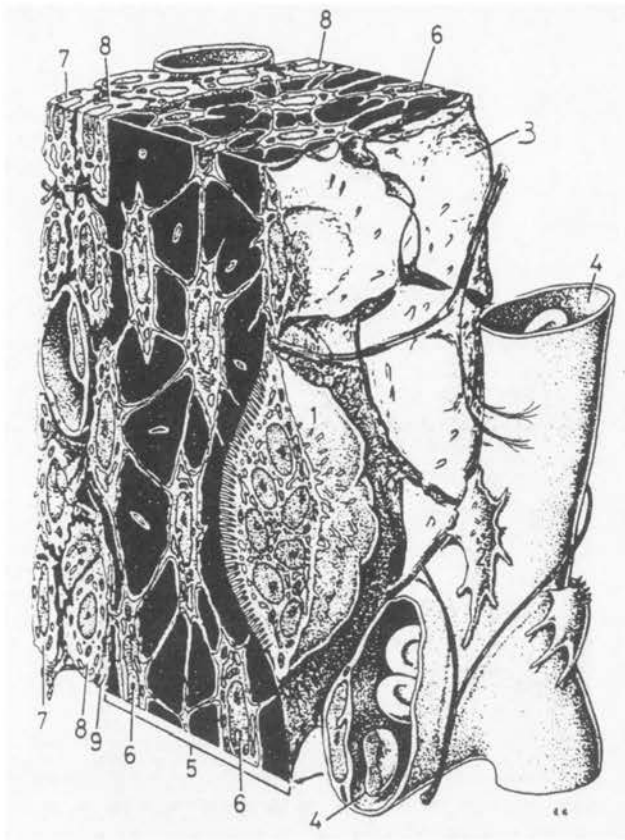
FIGURE 1. Diagram of a thin bone trabecula (5) showing the four types of bone cells. Osteoblasts (8) and their precursors (7) are shown on the upper surface over a layer of uncalcified osteoid matrix (9), osteocytes (6) are shown in their lacunae, an osteoclast (1) and a bone lining cell (3) are shown on the lower surface. Capillaries (4), containing red blood cells in their lumina, and a fibroblast (2) are shown near the trabecula. Adapted from [194].

in Fig. 2. The walls of the channel consist of matching rings of proteins piercing the membrane of each cell, and when the rings associated with two cells connect with each other, the cell-to-cell junction is formed. This junction allows ions and compounds of low molecular weight to pass between the two cells without passing into the extracellular space. The proteins making up a gap junction are called connexins; in bone the protein is either connexin 43 or 45, with 43 predominating (the number refers to the size of the proteins calculated in kilodaltons) [31, 32]. A ring of connexins in one cell membrane is generally called a connexon or hemichannel. Both mechanical strain and fluid shear stress cause increased expression of the connexin 43 in vascu-
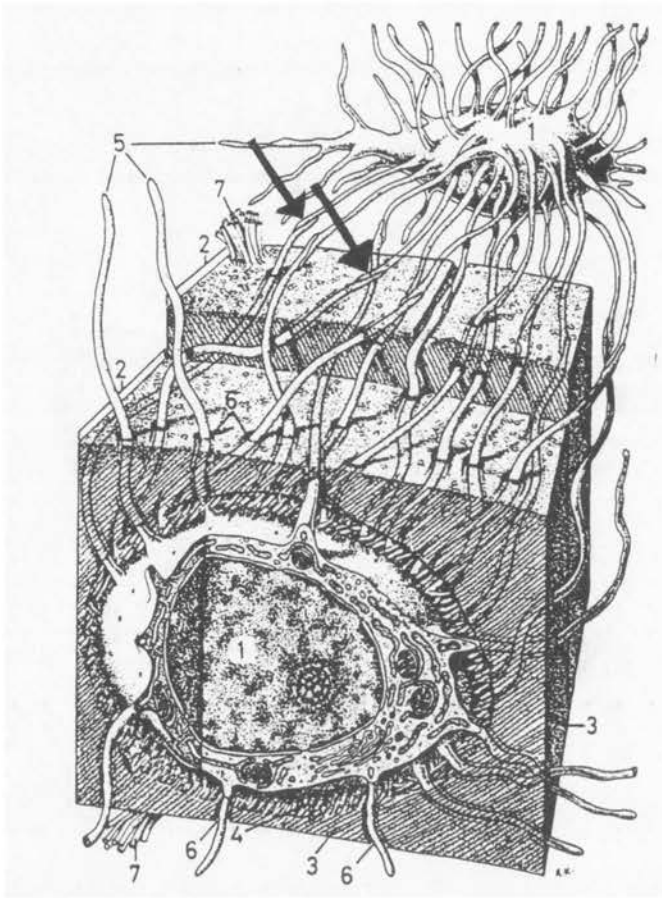
FIGURE 2. Diagram of two osteocytes (1) in the lamellar bone of calcified bone matrix (3). Two neighboring lamellae (2) with different collagen fiber orientations (7) are visible. The osteocytic cell bodies are located in lacunae and are surrounded by a thin layer of uncalcified matrix (4). Their cell processes (5) are housed in canaliculi (6). Some of the gap junctions between the cell processes are indicated (arrows). Modified from [194].

lar tissues [33]. In cardiac tissue the turnover rate of connexin 43 is very rapid [34]. The rapid dynamics of gap junction turnover and the plasticity of gap junction expression in response to various stimuli offer the possibility for remodeling of the intercellular circuits both within and between communication compartments in the cardiovascular system [35]. In bone, gap junctions connect superficial osteocytes to periosteal and endosteal osteoblasts. All osteoblasts are similarly interconnected laterally on a bony surface; perpendicular to the bony surface, gap junctions connect periosteal osteoblasts

Lacunae

Canaliculi

This quasi-circular arc is part of the cellular interface (IC).

Space for a blood vessel or nerve

Cement line

Osteonal Canal

The collagen-apatite porosity exists in the domains outside of the vascular and lacunar-canalicular porosities (PV & PLC).

The surfaces of the lacunae and the canaliculi form the lacunar-canalicular interface (ILC).
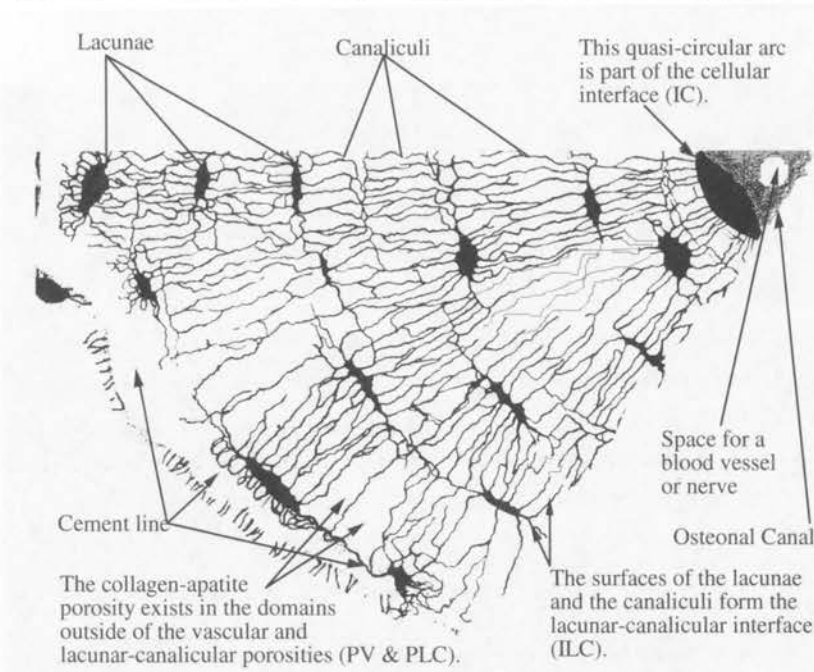
FIGURE 3. A pie-shaped section of an osteon. The osteonal canal is on the upper right, the cement line to the left. The osteonal canal is part of the vascular porosity (PV), the lacunae and the canaliculi are part of the lacunar-canalicular porosity (PLC) and the material in the space that is neither PV or PLC contains the collagen-apatite porosity (PCA).The three interfaces, the cement line, the cellular interface (IC) and the lacunar-canalicular interface are each indicated. The radius of an osteon is usually about 100 μm, and the long axis of a lacuna is about 15 μm. Using this information it should be possible to establish the approximate scale of the printed version of this illustration. Previously published in [93].

with preosteoblastic cells, and these, in turn, are similarly interconnected. Effectively, each CCN is a true syncytium [25, 27, 28, 36]. Gap junctions are found where the plasma membranes of a pair of markedly lapping canalicular processes meet [36]. In compact bone, canaliculi cross the cement lines that form the outer boundary of osteons. Thus extensive communication exists between osteons and interstitial regions [37].

Bone cells are electrically active [38, 39, 40, 41, 42]. In addition to permitting the intercellular transmission of ions and small molecules, gap junctions exhibit both electrical and fluorescent dye transmission [43, 44, 45, 46]. Gap junctions are electrical synapses, in contradistinction to interneuronal, chemical synapses; and, significantly, they permit bi-directional signal traffic (e.g., biochemical, ionic, electrical etc.). In a physical sense, the CCN represents the hard wiring [20, 21, 22, 47] of bone tissue.
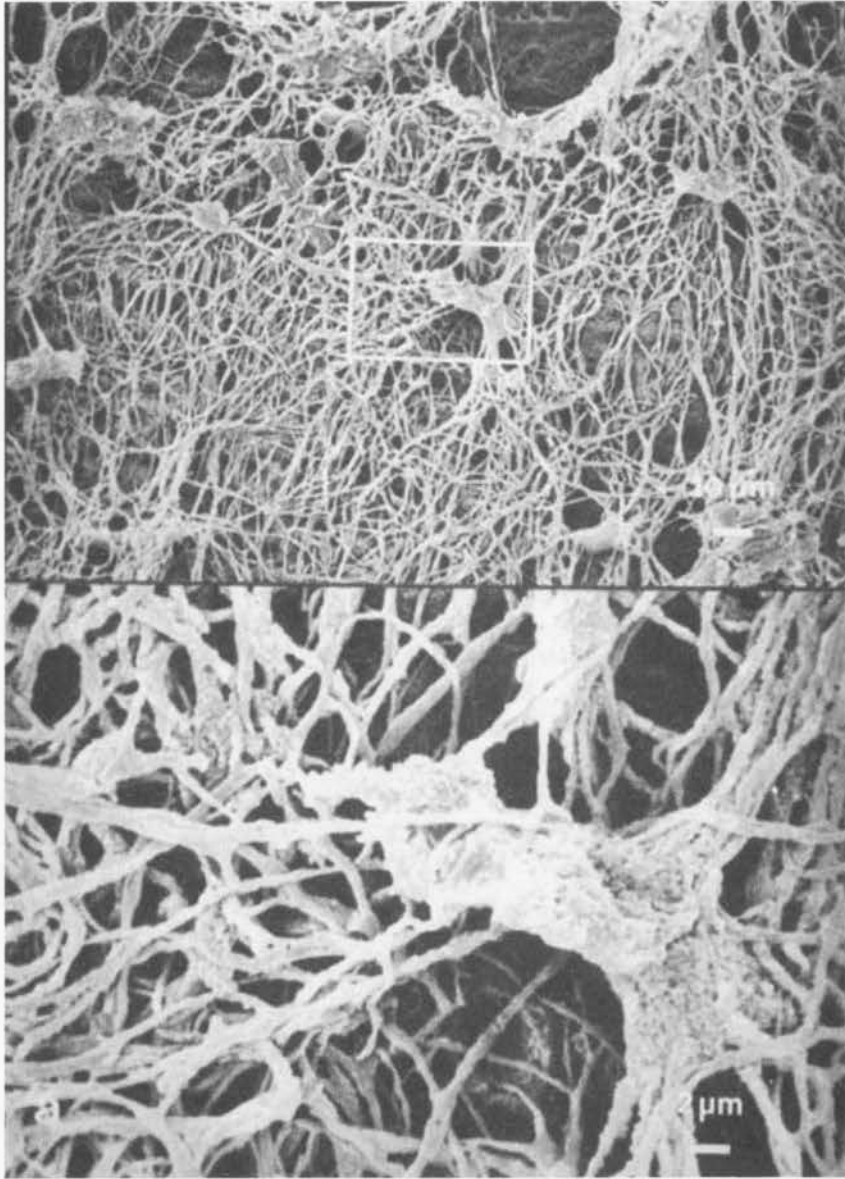
FIGURE 4. A scanning electron micrograph showing the replicas of lacunae and canaliculi *in situ* in mandibular bone from a young subject aged 22 years. The inset shows enlarged lacunae identified by a rectangle. This micrograph illustrates the interconnectivity of the connected cellular network (CCN). Copied from [195].
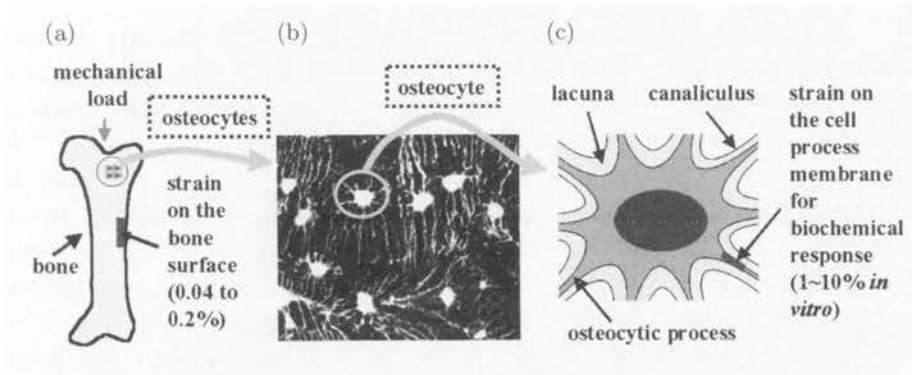
FIGURE 5. The paradox addressed in mechanosensation. (a) An illustration of the small strains that the whole bone experiences, strains that are in the range 0.04 to 0.3 percent and seldom exceed 0.1 percent. The last two panels, (b) Photomicrograph of osteocytes encased in bone matrix (c) Osteocyte in lacuna, illustrate that large strains (1 to 10 percent) on cell membrane are needed to induce biochemical intracellular response *in vitro*. The paradox in the bone mechanosensing system is that the strains that activate the bone cells are two orders of magnitude larger than the strains to which the whole bone organ is subjected. Previously published in [67].

3. Mechanosensation in bone: stimuli

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 bone tissue domain or region over which the stimulus is felt is called the *sensor* domain. When an appropriate stimulus parameter exceeds threshold values, loaded tissues respond by the triad of bone adaptation processes: deposition, resorption and maintenance. The CCN is the site of intracellular stimulus reception, signal transduction and intercellular signal transmission. It is thought that stimulus reception occurs in the osteocyte [20], and that the CCN transduces and transmits the signal to the surface lining or osteoblast. The osteoblasts alone directly regulate bone deposition and maintenance, and indirectly regulate osteoclastic resorption [48]. The possible role of the osteoblast as a stimulus receptor has not yet been thoroughly investigated [49]. Although it is reasonably presumed that initial mechanosensory events occur at the plasma membrane of the osteocytic soma and/or canalicular processes, the initial receptive, and subsequent transductive, processes are not well understood.

It follows that the true biological stimulus, although much discussed, is not precisely known. A variety of mechanical loading stimuli associated

with ambulation (at a frequency of one to two Hz) have been considered for bone remodeling. The majority has followed Wolff [2, 3] in suggesting that some aspect of the mechanical loading of bone is the stimulus. The mechanical stimuli suggested include strain [50], stress [2, 3], strain energy [51, 52], strain rate [53, 54, 55, 56, 57, 58, 59], and fatigue microdamage [60, 61]. In some cases the time-averaged values of these quantities are suggested as the mechanical stimulus, and in others the amplitudes of the oscillatory components and/or peak values of these quantities are the candidates for the mechanical stimulus. Two-dozen possible stimuli were compared in a combined experimental and analytical approach [62]. The data supported strain energy density, longitudinal shear stress and tensile principal stress or strain as stimuli; no stimulus that could be described as rate dependent was among the two dozen possible stimuli considered in the study. For a consideration of the stimulus in microgravity see [63].

The case for strain rate as a remodeling stimulus has been building over the last quarter century. The animal studies of Hert and his coworkers [53, 54, 55] suggested the importance of strain rate. Experiments [57, 58, 59] have quantified the importance of strain rate over strain as a remodeling stimulus. The studies [64, 65, 66] 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 whose signal is transduced by osteocytes. In the model [64, 65, 66] developed, the shear stress from the bone fluid flow over the osteocytic processes in the canaliculi is a cellular mechanism-based model suggesting strain rate as a stimulus. A later study [67] showed that it was not shear stress applied directly to the cell membrane, but rather the force created by fluid drag on the fibers of the glycocalyx of the cell transmitted to the cell membrane. This study will be described in grater detail in the section on strain amplification. A recent study [68] that showed bone deposition to be related to strain gradients actually demonstrates a dependence upon strain rate if the model developed in [64, 65, 66] is realistic.

In experiments with cultured cells 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 [69, 70]. Three different cell populations, namely osteocytes, osteoblasts, and periosteal fibroblasts, were subjected to two stress regimes, pulsatile fluid flow and intermittent hydrostatic compression [69]. Intermittent hydrostatic compression was applied at 0.3 Hz with a 13 kPa peak pressure. The pulsatile fluid flow was a fluid flow with a mean shear stress of 0.5 Pa with cyclic variations of 0.02 Pa at 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 1 hour pulsatile fluid flow treatment with a sustained prostaglandin E_2 upregulation lasting at least one hour after pulsatile fluid flow was terminated. By comparison, IHC needed 6 hours treatment before a response was found. 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. These conclusions are in agreement with the theory that osteocytes are the main mechanosensory cells of bone, and that they detect mechanical loading events by the canalicular flow of interstitial fluid which results from that loading event. The model developed in [64, 65, 66] used Biot's porous media theory to relate loads applied to a whole bone to the flow of canalicular interstitial fluid past the osteocytic processes. These calculations predicted 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 [69, 70, 71, 72, 73] lend experimental support to the model [64, 65, 66].

Osteocytes also rapidly release nitric oxide in response to stress [70, 74] and this NO response seems to be required for the stress-related prostaglandin release [70]. Therefore, the behavior of osteocytes compares to that of endothelial cells which 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 [75]. 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 [76].

Skeletal muscle contraction is a typical bone-loading event and has been suggested [77, 78, 79] as a stimulus. 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 [80]. It has been observed [81, 82] that these higher order frequencies, significantly related to bone adaptational responses, are [83] "... present within the [muscle contraction] strain energy spectra regardless of animal or activity and implicate the dynamics of muscle contraction as the source of this energy band." The close similarity of muscle stimulus frequencies to bone tissue response frequencies is noted below.

4. Strain amplification

There is a fundamental paradox in the physiology of bone mechanosensation. The paradox (Fig. 6) 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 [59, 84]. Osteocytes (Fig. 2) are believed to be the critical mechanical sensor cells [20, 85], although the mechanism by which osteocytes perceive mechanical load is not known. One widely held idea is that cell membrane stretch occurs as a direct result of surrounding tissue deformation. If this is the case, then strain on osteocyte membranes should be comparable to the bone tissue strain. However, in *vitro* studies show that in order to induce any cellular response by direct mechanical deformation of bone cells, deformations need to be one to two orders of magnitude larger than the bone tissue strains normally experienced by the whole bone *in vivo* [67, 86]. Similar cell strain magnitudes are needed to activate fibroblasts and chondrocytes (~15%) [87, 88], suggesting that in their sensitivity to mechanical strain, osteocytes may not be different from other connective tissue cells. However, in bone the larger strains needed to stimulate osteocytes cannot be derived directly from matrix deformations, as they would cause bone fracture. Thus, in bone there is an inherent contradiction between material and biological stimulation requirements. A hypothesis and model to deal with this contradiction is given in [67].

The flow of bone fluid due to mechanical loading through the lacuno-canalicular system is an important aspect of the model presented in [67]. Between the osteocyte cell process membrane and canalicular wall is the pericellular space through which the bone fluid flows (Figs. 2 and 3). A pericellular organic matrix appears to fill the space [89, 90]. This matrix is supported by transverse fibrils [91] that appear to anchor and center the cell process in its canaliculus. When a whole bone is deformed, the deformation-induced pressure gradient will cause bone fluid to flow in the pericellular space of the lacunar-canalicular system [64, 65, 66, 92, 93, 94] and induce a drag force on the matrix fibers.

The effect of fluid drag forces on the pericellular matrix and its coupling to the intracellular actin cytoskeleton and the strain amplification that results from this coupling, was examined in [67]. The fluid drag on an attached pericellular matrix causes a circumferential (hoop) strain in the membrane-cytoskeleton of the cell process. The model in [67] shows that, for the loading range 1 to 20 MPa and frequency range 1-20 Hz, it is, indeed, possible to produce cellular level strains in bone that are up to 100 fold greater than normal tissue level strains (0.04% to 0.3%). Thus, the strain in the cell process
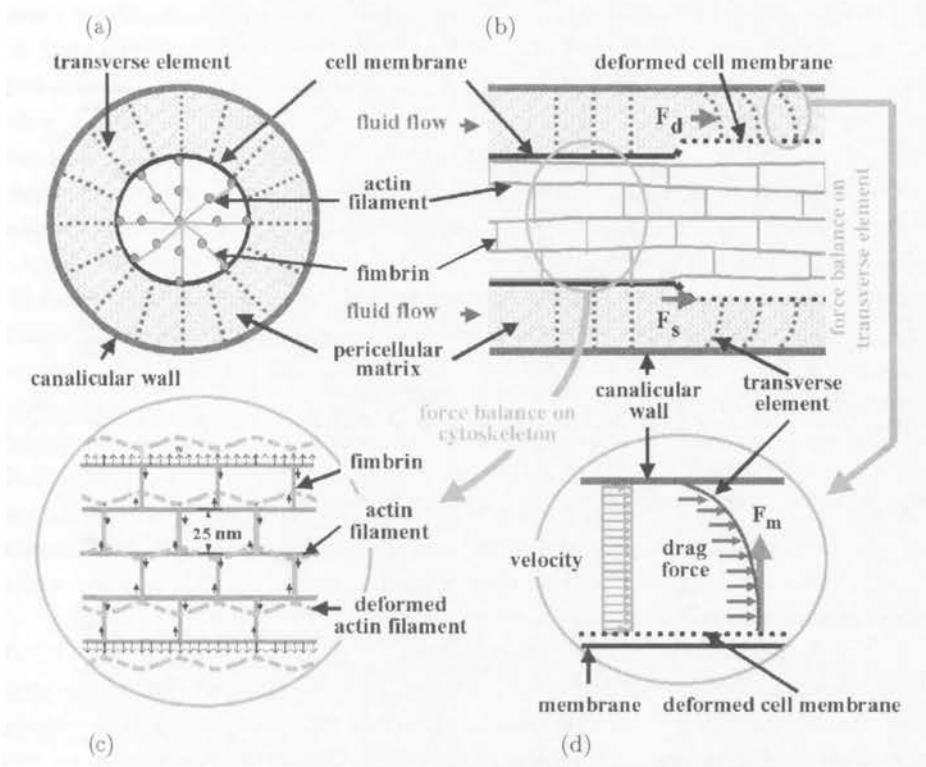
FIGURE 6. Schematic model showing the structure of the PM, the intracellular actin cytoskeleton inside the process and the connection between the PM and the IAC. (a) transverse cross-section of canaliculus showing the fluid annular shape of the region and transverse (radial) pericellular fibers. (b) longitudinal cross-section before and after the transverse elements are deformed by the flow. (c) schematic of the cell process cytoskeletal structure in longitudinal axial section used to estimate the Young's modulus in the radial (vertical) direction. Since the length of the cell process is 300 times its radius, it is considered infinite in the longitudinal (horizontal) direction. The axial actin filaments shown are modeled as continuous infinite beams with two types of loadings depending on whether the actin filaments are peripheral or interior. The small vertical arrows indicate the direction of the loading. The (fimbrin) links between these infinitely long beams are considered to be rigid. (d) Force balance on a transverse element. Previously published in [67].

membrane due to the loading can be of the same order as the *in vitro* strains measured in cell culture studies where intracellular biochemical responses are observed for cells on stretched elastic substrates.

An idealized model for an individual canaliculus with its central cell process is a tube containing a centrally positioned osteocyte process and its

surrounding fluid annulus filled with a mesh-like pericellular matrix is shown in Fig. 6. For the pericellular component, only two structural elements are critical for this mechanical model: 1) a space filling pericellular matrix with a fiber spacing Δ that is sufficiently small, and 2) transverse fibrils which tether the cell process to the canalicular wall. From a mechanics point of view, any matrix, which has these two characteristics, should function equivalently, although the degree of strain amplification will change with the fiber spacing Δ. There is growing evidence to support this basic structure. First, a space filling pericellular matrix surrounding osteocytes is well-established [89, 90, 91, 95, 96]. Second, transverse tethering elements were first clearly identified in Fig. 3 in [91]. The pericellular space surrounding the osteocyte process varies from 14 nm to 100 nm [97, 98, 99], depending on species, age, age of osteocyte, histological bone type, skeletal location, etc. As yet unreported EM studies by Lidan You on adult mice indicate a pericellular space of 30-50 nm. Lidan You also observed that the cell process is invariably located at the center of the canalicular cross-section suggesting that the transverse fibrils are tension elements that anchor and position the cell process within the canaliculus.

Both albumin and proteoglycan exist in the pericellular space [89, 100]. The effective diameter of albumin is approximately 7 nm, similar to the spacing of glycosaminoglycans (GAG) side chains along a proteoglycan monomer [101]. The model of Weinbaum and Cowin [64, 65, 66] suggested that the value of pore size leads to shear stresses of 0.5 to 3.0 Pa for mechanical loads in the physiological range and good agreement with the experimental data for the relaxation time of stress generated potentials (SGP) in bone under four point bending [102, 103, 104]. A pericellular matrix with a most likely pore size of 7 nm was assumed for the model in [67].

Finally, the matrix must be attached to the cell process and the canalicular wall in order for the drag force to be transmitted to the membrane and its underlying intracellular actin cytoskeleton. If such linker molecules are present, drag forces exerted on the matrix fibers will produce a tensile stress on these linker molecules that, in turn, will produce radial (hoop) strain in the intracellular actin cytoskeleton as schematically shown in Fig. 6. Possible candidates for these attachment molecules are CD44, laminin, and various integrins [105, 106].

Osteocyte processes contain a space filling actin bundle [99, 107], whose actin filaments are cross-linked at regular intervals along the axis of the process by a linker molecule recently identified as fimbrin [107] as shown in Fig. 6(c). The axial actin filaments are $\sim$ six nm in diameter. Fimbrin is also found in intestinal microvilli [108] as well as non-intestinal cell microvilli [109]. The typical spacing of fimbrin cross-linked actin filaments in

microvilli is approximately 25 nm [110]. This spacing is consistent with the EM observation in [99] and [107] for an osteocyte process.

The strain amplification ratio ε_r is defined as the ratio of the hoop strain in the cell process membrane to the bone surface strain at the osteonal lumen. The effect of frequency on the strain amplification ratio at different loading magnitudes is shown in Fig. 7, where the strain amplification ratio is plotted against the loading frequency from 1 to 20 Hz. The hoop strain is calculated at the position of maximum flow or pressure gradient, which is located at the surface of the Haversian canal. Thus, the strain amplification ratio shown in Fig. 7 can be considered as an upper bound. The curves show a monotonic increase in the amplification ratio as a function of frequency for a prescribed loading. One observes that the amplification ratio varies from 19 to 122 and depends significantly on the magnitude of the loading. When the loading magnitude is one MPa, corresponding to 50 μstrain at the osteonal lumen, the strain amplification ratio ε_r is 122 at 20 Hz. For this loading the cell process strain is 0.68 percent. For a 20 MPa load at 20 Hz, $\varepsilon_r = 44$ and the cell process strain is 4.9 percent. The corresponding values of ε_r at 1 Hz for a 1 and 20 MPa load are 51 and 19 and the corresponding strains are 0.29 and 2.1 percent, respectively. Strains of the order of 0.3 percent or greater fall in the range where cellular level biochemical responses have been observed *in vitro* in four point bending [74].
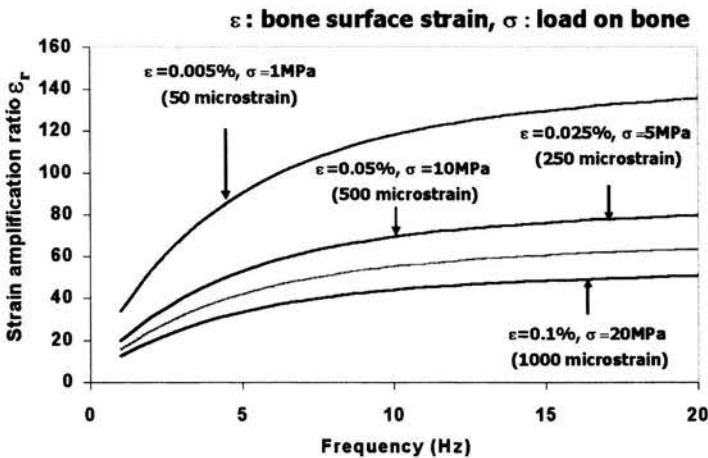

ε: **bone surface strain, σ : load on bone**

FIGURE 7. Strain amplification: A plot of the strain amplification ratio ε_r as a function of the load frequency for different load magnitudes. Strain amplification ratio is defined as the ratio of the hoop strain in the cell process membrane to the bone surface strain at the osteonal lumen. ε is the strain on the whole bone; σ is the load on the whole bone. Previously published in [67].

5. Mechanosensation in bone: reception and transduction

The osteocyte has been suggested as the stimulus sensor, the receptor of the stimulus signal [20]; histologic and physiologic data are consistent with this suggestion [90, 96, 111]. The placement and distribution of osteocytes in the CCN three-dimensional array is architecturally well suited to sense deformation of the mineralized tissue encasing them [112]. Since only a population of cells, and not an individual receptor [113], can code unambiguously, the osteocytes in the CCN are potential mechanoreceptors by virtue of their network organization.

Osteocytic mechanotransduction may involve a number of different processes or cellular systems. These processes include stretch- and voltage-activated ion channels, cyto-matrix sensation-transduction processes, cyto-sensation by fluid shear stresses, cyto-sensation by streaming potentials and exogenous electric field strength. Each of these processes or cellular systems is discussed below.

5.1. Stretch- and voltage-activated ion channels

The osteocytic plasma membrane contains stretch-activated ion channels [11, 114, 115, 116, 117, 118, 119] that are also found in many other cell types [120, 121]. When activated in strained osteocytes, they permit passage of certain ions (Sachs 1986, 1988; Sackin, 1995), including K^+, Ca^{2+}, Na^+ and Cs^+. Such ionic flow may, in turn, initiate cellular electrical events; e.g., bone cell stretch-activated channels may modulate membrane potential as well as Ca^{2+} ion flux [11, 122]. Rough estimates of osteocytic mechanoreceptor strain sensitivity have been made [20], and the calculated values cover the morphogenetically significant strain range of 0.1% to 0.3% in the literature [57, 84, 123]. This appears to be too low a strain to open a stretch-activated ion channel.

As in most cells, the osteocytic plasma membrane contains voltage-activated ion channels, and transmembrane ion flow may be a significant osseous mechanotransductive process [40, 124, 125, 126]. It is also possible that such ionic flow generates osteocytic action potentials, capable of transmission through gap junctions [44].

5.2. Cyto-matrix sensation-transduction processes

The mineralized matrix of bone tissue is strained when loaded. Macromolecular mechanical connections between the extracellular matrix and the osteocytic cell membrane exist and these connections may be capable of transmitting information from the strained extracellular matrix to the bone

cell nuclear membrane. The basis of this mechanism is the physical continuity of the transmembrane integrin molecule, which is connected extracellularly with the macromolecular collagen of the organic matrix and intracellularly with the cytoskeletal actin. The latter, in turn, is connected to the nuclear membrane [91, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139]. It is suggested that such a cytoskeletal lever chain, connecting to the nuclear membrane, can provide a physical stimulus able to activate the osteocytic genome [26], possibly by first stimulating the activity of such components as the *c-fos* genes [26, 128, 135, 140, 142, 143, 144, 145, 146, 147, 148].

5.3. Cyto-sensation by fluid shear stresses

An hypothesis concerning the mechanism by which the osteocytes housed in the lacunae of mechanically loaded bone sense the load applied to the bone by the detection of dynamic strains was suggested in [64, 65, 66]. 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 that 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 in [64, 65, 66]. 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 would 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 nuclear membrane, where it can effectively regulate its genomic functions.

Several investigators [92, 147, 149] have examined other aspects of the lacunar-canalicular porosity using simple circular pore models and have attempted to analyze its possible physiological importance. These studies have primarily emphasized the importance of the convective flow in the canaliculi between the lacunae as a way of enhancing the supply of nutrients between neighboring osteocytes. Previous studies on the relaxation of the excess pore pressure have been closely tied to the strain generated potentials (SGP) associated with bone fluid motion. The SGP studies are briefly reviewed below.

5.4. The cyto-sensation is actually caused by fluid drag rather than fluid shear

The model of You *et al.* discussed in the previous section was used to make a second interesting prediction. It demonstrated that in any cellular system where cells are subject to fluid flow and tethered to more rigid supporting structures, the tensile forces on the cell due to the drag forces on the tethering fibers may be many times greater than the fluid shear force on the cell membrane. As proposed in [64, 65, 66] the fluid flow will also induce shear stress on the cell process membrane. These stresses have been shown to mechanically stimulate bone cells [71, 72]. In the case of the extension [67] of the model proposed in [64, 65, 66] for fluid flow in the canaliculus, the drag force on the matrix is larger than the fluid shear force. The matrix must be attached to the cell process and the canalicular wall in order for the drag force to be transmitted to the membrane and its underlying intracellular actin cytoskeleton.

The force ratio F_r is defined as the ratio of the drag force on the fibers to the shear force on the cell process membrane per unit length of cell process. The relationship between the force ratio F_r and the average fiber spacing Δ is plotted in Fig. 8. Note the force ratio at $\Delta = 7\,\text{nm}$ is 19.6. $\Delta = 7\,\text{nm}$ is typical of the average spacing of GAG side chains along a core protein and the effective diameter of the albumin molecule that is known to be sieved by an equivalent matrix in capillary endothelium. This varies between 5 and 12 nm.

FIGURE 8. The relationship between force ratio F_r and average fiber spacing Δ. Note the force ratio at $\Delta = 7\,\text{nm}$ is 19.6. $\Delta = 7\,\text{nm}$ is typical of the average spacing of GAG side chains along a core protein and the effective diameter of the albumin molecule which is known to be sieved by an equivalent matrix in capillary 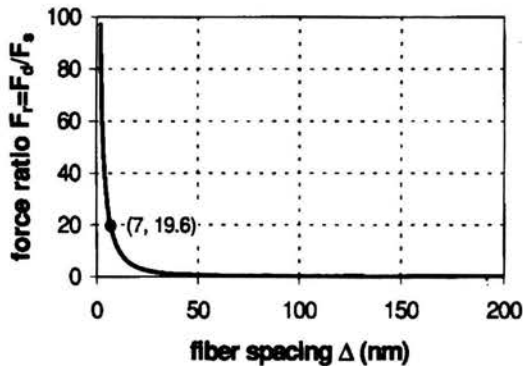endothelium. This varies between 5 and 12 nm. The force ratio F_r is defined as the ratio of the drag force on the fibers to the shear force on the cell process membrane per unit length of cell process. Previously published in [67].

5.5. Cyto-sensation by streaming potentials

The fact that the extracellular bone matrix is negatively charged due to its proteins means that a fluid electrolyte bounded by the extracellular matrix will have a diffuse double layer of positive charges. When the fluid moves, the excess positive charge is convected, thereby developing streaming currents and streaming potentials (Fig. 9). The cause of the fluid motion is the deformation of the extracellular matrix due to whole bone mechanical loading. Pollack and coworkers [102, 103, 150] have laid an important foundation for explaining the origin of strain-generated potentials (SGP). However the anatomical site in bone tissue that is the source of the experimentally observed SGPs is not agreed upon. It was concluded in [103] that this site was the collagen-hydroxyapatite porosity of the bone mineral, because small pores of approximately 16 nm radius were consistent with their experimental data if a poroelastic-electrokinetic model with unobstructed and connected circular pores was assumed [102]. However in [66] it was shown that the data presented in [103, 104, 151] are also consistent with the larger pore space (100 nm) of the lacunar-canalicular porosity being the anatomical source site of the SGPs if the hydraulic drag and electrokinetic contribution associated
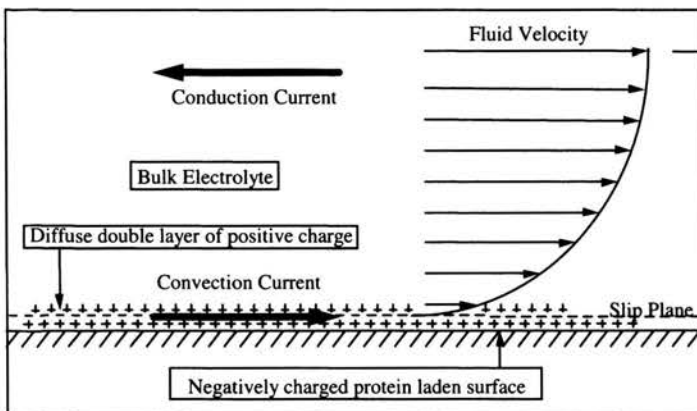

FIGURE 9. Strain-generated potentials (SGP's) in bone fluid channels. The source of SGP's stems from the fact that the extracellular bone matrix is negatively charged due to negative fixed charges on carbohydrates and proteins; thus a fluid electrolyte bounded by the extracellular matrix will have a diffuse double layer of positive charges (see also Figure 10.10). When the fluid moves, the excess positive charge is convected, thereby developing streaming currents and streaming potentials. A conduction current is thought to balance the convection current. The bulk electrolyte is neutral with respect to charge. The fluid motion is caused by the pore fluid pressure gradients induced by the deformation of the extracellular matrix due to whole bone mechanical loading.

with the passage of bone fluid through the surface matrix (glycocalyx) of the osteocytic process are accounted for. The mathematical models presented in [102] and [64, 65, 66, 67] are similar in that they combine poroelastic and electrokinetic theory to describe the phase and magnitude of the SGP. The two theories differ in the description of the interstitial fluid flow and streaming currents at the microstructural level and in the anatomical structures that determine the flow. In [64, 65, 66] this resistance resides in the fluid annulus that surrounds the osteocytic processes, i.e. the cell membrane of the osteocytic process, the walls of the canaliculi and the glycocalyx (also called the surface matrix or capsule) that exists in this annular region. In [66] the presence of the glycocalyx increases the SGPs and the hydraulic resistance to the strain-driven flow. The increased SGP matches the phase and amplitude of the measured SGPs. In the [102] model this fluid resistance and SGP are achieved by assuming that an open, continuous small pore structure ($\approx 16\,\text{nm}$ radius) exists in the mineralized matrix. The poroelastic model of [64, 65, 66] for bone fluid flow has been developed further [152, 153, 154, 155, 156] and a review of the related poroelastic literature has appeared [93].

Experimental evidence indicating that the collagen-hydroxyapatite porosity of the bone mineral is unlikely to serve as the primary source of the SGP is obtained from several sources, including the estimates of the pore size in the collagen-hydroxyapatite porosity and permeability studies with different size labeled tracers in both mineralized and unmineralized bone. Such permeability studies clearly show time-dependent changes in the interstitial pathways as bone matures. At the earliest times, the unmineralized collagen-proteoglycan bone matrix is porous to large solutes. The studies with ferritin (10 nm in diameter) in two-day old chick embryo [157] show a continuous halo around primary osteons five minutes after the injection of this tracer. The halo passes right through the lacunar-canalicular system suggesting that, before mineralization, pores of the size predicted in [103] (radii $\approx 16\,\text{nm}$) can exist throughout the bone matrix. In contrast a report of an experiment [158] using this same tracer in adult dogs also found a fluorescent halo surrounding the Haversian canals; however this halo was not continuous but formed by discrete lines suggesting that the pathways were limited to discrete pores whose spacing was similar to that observed for canaliculi. This conclusion is supported by the studies [159] in the alveolar bone of five-day-old rats using the much smaller tracer, microperoxidase (MP) (2 nm). These studies clearly showed that the MP only penetrated the unmineralized matrix surrounding the lacunae and the borders of the canaliculi (see Fig. 13 of this study) and was absent from the mineralized matrix. Using more mature rats, the study of [160] confirmed the failure of the small (2 nm) MP tracer to penetrate the mineralized matrix tissue from the bone fluid compartments.

5.6. Exogenous electric field strength

Bone responds to exogenous electrical fields [161]. While the extrinsic electrical parameters are unclear, field strength may play an important role [161, 162]. A significant parallelism exists between the parameters of exogenous electrical fields and the endogenous fields produced by muscle activity. Bone responds to exogenous fields in an effective range of 1-$10\,\mu V/cm$, strengths that are on the order of those endogenously produced in bone tissue during normal (muscle) activity [163].

6. Mechanosensation in bone: Signal Transmission

From a communications viewpoint the CCN is multiply noded (each osteocyte is a node) and multiply connected (Fig. 4). Each osteocytic process is a connection between two osteocytes, and each osteocyte is multiply connected to a number of osteocytes that are near neighbors. Cell-to-cell communication is considered first below, then some speculative considerations of the ability of the CCN to compute as well as signal are described. It is useful to note the possibility that bone cells, like neurons, may communicate intercellular information by volume transmission, a process that does not require direct cytological contact, but rather utilizes charges in the environment [164, 165, 166].

6.1. Cell-to-cell communication

In order to transmit a signal over the CCN one osteocyte must be able to signal a neighboring osteocyte that will then pass the signal on until it reaches an osteoblast on the bone surface. There are varieties of chemical and electrical cell-to-cell communication methods [167]. 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. The focus here is on electrical cell-to-cell communication. A cable model for cell-to-cell communication in an osteon has been formulated [152, 153]. 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 (SGPs) produced by the cyclic mechanical loading of bone. The model differs from earlier studies [168] in that it pursues a more physiological approach in which the micro-anatomical dimensions of the connexon pores, osteocytic processes and the distribution

of cellular membrane area and capacitance are used to quantitatively esti-
mate the leakage of current through the osteoblast membrane, the time delay
in signal transmission along the cable and the relative resistance of the osteo-
cytic processes and the connexons in their open and closed states. The model
predicts that the cable demonstrates a strong resonant response when the ca-
ble coupling length approaches the osteonal radius. 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. These two resonances lead to a large amplification of the intracel-
lular potential and current in the surface osteoblasts, which could serve as
the initiating signal for osteoblasts to conduct remodeling.

6.2. Signal processing and integration

When a physical representation of a CCN, such as Fig. 1, is viewed by
someone familiar with communications there is often an intuitive response
that the CCN may function as a neural network for processing the mechani-
cal loading stimulus signals being felt over the network. That idea is explored
here with no justification other than shared intuition. A CCN is operationally
analogous to an artificial neural network in which massively parallel, or par-
allel distributed, signal processing occurs [113, 169, 170]. Fortunately the
bases of connectionist theory are sufficiently secure to permit a biologically
realistic CCN model [171, 172, 173, 174, 175].

A CCN consists of a number of relatively simple, densely interconnected,
processing elements (bone cells), with many more interconnections than cells.
Operationally these cells are organized into layers: an initial input, a final
output and one or more intermediate or hidden layers. However such networks
need not be numerically complex to be operationally complex [176].

The operational processes are identical, in principle, for all bone cells in
all layers. Each cell in any layer may simultaneously receive several weighted
(i.e., some quantitative measure) inputs. In the initial layer these are the
loading stimuli (mechanoreception). Within each cell independently, "... all
the weighted inputs are then summed..." [177]. This net sum is then com-
pared, within the cell, against some threshold value. If this liminal value is
exceeded, a signal is generated (mechanotransduction in input layer cells)
that is then transmitted identically to all the hidden layer cells (adjacent
osteocytes) to which each initial layer cell is connected. Similar processes
of weighted signal summation, comparison and transmission occur in these
layers until final layer cells (osteoblasts) are reached. The outputs of these

surface situated cells regulate the specific adaptation process of each group of osteoblasts [178]. All neighboring osteoblasts that carry out an identical bone adaptational process form a communication compartment, a cohort of structurally and operationally similar cells, since all these cells are interconnected by open, functional gap junctions. At the boundary between such compartments that are carrying out different adaptational processes, the intervening gap junctions are closed and are incapable of transmitting information. These boundaries are probably changing continuously as some of the cells have some down time [35, 43].

Information is not stored discretely in a CCN, as is the case in conventional computers. Rather it is distributed across all or part of the network, and several types of information may be stored simultaneously. The instantaneous state of a CCN is a property of all of its cells and of their connections. Accordingly its informational representation is redundant, assuring that the network is fault, or error, tolerant; i.e., one or several inoperative cells causes little or no noticeable loss in network operations [177].

CCNs exhibit oscillation; i.e., iterative reciprocal (feedback) signaling between layers enables them to adjustively self-organize. This is related to the fact that CCNs are not preprogrammed, rather they learn by unsupervised training [179], a process involving the adaptation of the CCNs to the responses of the cytoskeleton to physical activity [141]. In this way the CCN adjusts to the customary mechanical loading of the whole bone [57]. In a CCN, structurally more complex attributes and behavior gradually self-organize and emerge during operation. These are not reducible; they are neither apparent nor predictable from a prior knowledge of the behavior of individual cells.

As noted above, gap junctions as electrical synapses permit bi-directional flow of information. This is the cytological basis for the oscillatory behavior of a CCN. The presence of sharp discontinuities between groups of phenotypically different osteoblasts is related also to an associated property of gap junctions, i.e., their ability to close and so prevent the flow of information [180, 181]. Significantly, informational networks can also transmit inhibitory signals, a matter beyond our present scope [182].

It is suggested that a CCN displays the following attributes: Developmentally it is self-organized and self-adapting and, in the sense that it is epigenetically regulated, it is untrained. Operationally it is a stable, dynamic system, whose oscillatory behavior permits feedback; in this regard, it is noted that a CCN operates in a noisy, non-stationary environment, and that it also employs useful and necessary inhibitory inputs.

The CCN permits a triad of histological responses to a (seemingly) unitary loading event. Although in this chapter, as in almost all the related

literature, the organization of bone cells is treated as if it existed only in two dimensions, and as if bone tissue loadings occurred only at certain discrete loci, and that without consideration of loading gradients, the biological situation is otherwise. Given such a loading event, a three-dimensional bone volume, gradients of deformation must exist, and each osteocyte may sense correspondingly different strain properties. Moreover, it is probable that each osteocyte potentially is able to transmit three different signals in three different directions, some stimulatory and some inhibitory: such states have not yet been modeled [183, 184].

6.3. A tentative mechanotransduction synthesis

The molecular lever mechanisms that permit muscle function to directly regulate the genomic activity of strained bone cells, including their phenotypic expression, when combined together with electric field effects and contraction frequency energetics, provides a biophysical basis for an earlier hypothesis of epigenetic regulation of skeletal tissue adaptation [185, 186, 187,188].

It is probable [189, 190] that electrical and mechanical transductive processes are neither exhaustive nor mutually exclusive. While utilizing differing intermediate membrane mechanisms and/or processes, they share a common final pathway [191]; i.e., both mechanical and electrical transductions result in transplasma membrane ionic flow(s), creating a signal(s) capable of intercellular transmission to neighboring bone cells via gap junctions [64, 65, 66, 152, 153, 192]. These signals are inputs to a CCN, whose outputs regulate the bone adaptational processes.

The primacy of electrical signals is suggested here, since while bone cell transduction may also produce 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 [11, 17, 193]. As 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 30 Hz signal associated with muscle firing.

7. Mechanosensation in the auditory system

Hearing works by the ear detecting sound waves, converting them into neural signals and then sending the signals to the brain.[1] The ear has three

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[1] The simple explanation of the process of hearing that follows is adapted from the website http://www.bcm.tmc.edu/oto/research/cochlea/Hearing/index.html associated with John S. Oghalai, M.D. in Otolaryngology at the Baylor College of Medicine.
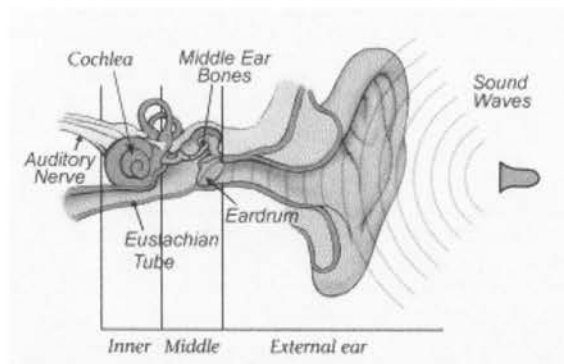
FIGURE 10. Anatomy of the ear. This figure is adapted from one on the website http://www.bcm.tmc.edu/oto/research/cochlea/Hearing/index.html associated with John S. Oghalai, M.D. in Otolaryngology at the Baylor College of Medicine.

divisions: the external ear, the middle ear, and the inner ear (Fig. 10). The external ear collects sound waves and funnels them down the ear canal, where they vibrate the eardrum. Within the middle ear, the eardrum is connected to the middle ear bones. These are the smallest bones in the body, and they mechanically carry the sound waves to the inner ear. The eustachian tube connects the middle ear to the upper part of the throat, equalizing the air pressure within the middle ear to that of the surrounding environment. The inner ear contains the cochlea. This organ converts sound waves into neural signals. These signals are passed to the brain via the auditory nerve.

Mechanosensation occurs in the cochlea (Fig. 10). A section through the cochlea is shown in Fig. 11. Coiling around the inside of the cochlea, the organ of Corti (Fig. 12) contains the cells responsible for hearing, the hair cells (Fig. 13). There are two types of hair cells: inner hair cells and outer hair cells. These cells have stereocilia or "hairs" that stick out. The bottom

FIGURE 11. An exposed cross-section of the cochlea illustrating its three chambers. Adapted from an illustration on the website
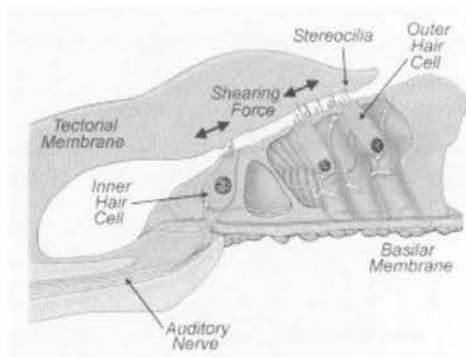http://www.finchcms.edu/anatomy/histohome/lectures/ear/sld001.htm.

FIGURE 12. The organ of Corti. The organ of Corti is the structure that partitions the chambers of the cochlea illustrated in Figure 11. This figure is adapted from one on the website `http://www.bcm.tmc.edu/oto/research/cochlea/Hearing/index.html` associated with John S. Oghalai, M.D. in Otolaryngology at the Baylor College of Medicine.
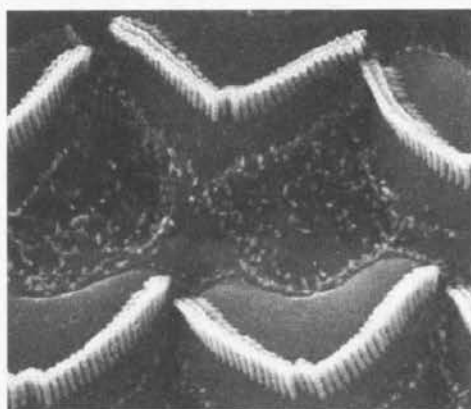

FIGURE 13. An array of hair cells.

of these cells is attached to the basilar membrane, and the stereocilia are in contact with the tectorial membrane. Inside the cochlea, sound waves cause the basilar membrane to vibrate up and down. This creates a shearing force (Fig. 12) between the basilar membrane and the tectorial membrane, causing the hair cell stereocilia to bend back and forth (Fig. 14). The movement of a hair cell's cilia bundle opens ion channels at the tips of the cilia (Fig. 14). When the bundle tilts to the right (middle panel of Fig. 14), tip links from the higher cilia pull up the gates of the ion channels on adjoining, shorter
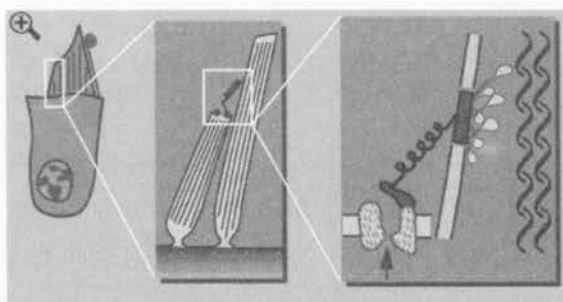
FIGURE 14. The movement of a hair cell's cilia bundle (left panel) opens ion channels at the tips of the cilia. When the bundle tilts to the right (middle panel), tip links from the higher cilia pull up the gates of the ion channels on adjoining, shorter cilia. This (still middle panel) close-up shows how a tip link between two cilia opens an ion channel on the shorter cilium. Even more highly magnified in the right panel, the open channel allows ions into the cell. This illustration is by Jennifer Jordan, RCW Communications, Inc. (adapted from a sketch by James Hudspeth, HHMI, University of Texas Southwestern Medical Center at Dallas).

cilia. A close-up shows how a tip link between two cilia opens an ion channel on the shorter cilium. Even more highly magnified at the right of Fig. 14, the open channel allows ions into the cell. This leads to internal changes within the hair cells that create electrical signals. Auditory nerve fibers rest below the hair cells and pass these signals on to the brain. Therefore, the bending of the stereocilia is how hair cells sense sounds.

Outer hair cells have a special function within the cochlea. They are shaped cylindrically, like a can, and have stereocilia at the top of the cell (Fig. 13), and a nucleus at the bottom. When the stereocilia are bent in response to a sound wave, an electromotile response occurs. This means the cell changes in length. Therefore, with every sound wave, the cell shortens and then elongates. This pushes against the tectorial membrane, selectively amplifying the vibration of the basilar membrane. This allows us to hear very quiet sounds.

The similarities between the cytoplasmic processes of bones cells and the stereocilia of hair cells are that they both (1) measure mechanical deformations (vibrations of a fluid domain), (2) communicate their measurement to a network, (3) do this with dendritic structures, (4) the dendrites of both cells are constructed of similar materials (e.g., actin and fimbrin) and (5) the initial signaling in both cases consists of opening ion channels. While the hair cells communicate their information to a network that feeds to the brain, the bones cells connect to a lower level network (CCN) with (potentially) local decision-making software.

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