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Morphological Evidence of Gap Junctions Between Bone Cells

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Summary. Cell membrane specializations occur at contact sites between adjacent osteoblasts and osteoblasts and osteocytes. These junctions have been described by other investigators as being important in preventing the extracellular movement of material around bone cells. Previously we described how certain small proteins circumvented the osteoblast population and rapidly penetrated the canalicular-osteocyte system. In the present study we used lanthanum colloid as an extracellular marker; the lanthanum readily penetrated the bone cell junctions and the extracellular space of bone. Morphologically, these junctions were not "tight" or "occluding" structures, but resembled "gap" junctions. These gap junctions contained elements which formed intercellular bridges between adjacent cells but also maintained a 2 nm space between cells that contained extracellular fluid. These gap junctions may have an important function in the control or coordination of bone cell activity throughout a given volume of bone.

Key words: Bone cells — Gap junctions — Cell communication.

When contact occurs between opposing cell membranes of adjacent osteoblasts, osteoblasts and osteocytes, or osteocyte processes, there is a structural modification at the membrane to form a special junction [1–5]. Some investigators described this modification as a "tight" or "occluding" junction, characterized by fusion of the outer leaflets of the opposing cell membranes [2–5]. The suggestion has been made that the junction is important as a barrier to prevent diffusion of extracellular material around bone cells [3]. Some reports [2, 4, 5] suggest that this junction plays a role in intercellular communication or transport in bone cells, although these studies provided no direct evidence to support such an idea.

Previously we have shown that extracellular tracers for ions or small proteins can move rapidly through the extracellular space of bone and penetrate the canalicular and osteocytic space within minutes after intravenous injection [1]. Thus, bone cells do not appear to form a physical barrier to the extracellular movement of all proteins.

If it can be shown that opposing bone cell membranes are not actually fused but retain a 2 nm extracellular space, then these junctions would be comparable to the "gap" junctions shown in other tissues which function in intercellular communication [6–10]. It has been shown that fluorescein dye can move intercellularly through adjacent bone cells after microinjection of the dye into only one osteoblast [11]. Such studies provide the physiological data needed to support the idea of a communication network between osteoblasts. In the present study we provide the morphological evidence that gap junctions and structures necessary for intercellular communication do exist between bone cells.

Materials and Methods

Bone tissue was obtained from Sprague-Dawley rats of various ages, and 1–2 mm^2 pieces of tibia and femur were placed in fixative. The fixative consisted of 4% glutaraldehyde in 0.1 M cacodylate buffer, mixed 1:1 with 4% aqueous lanthanum nitrate, and the final pH adjusted to 7.7–7.9 [12]. There was a barely visible colloidal precipitate in the fixative prior to adding the tissues. Fixation was carried out at room temperature for 3–6 h. Post-fixation was for 2 h at room temperature using 4% osmium tetroxide in 0.1 M veronal-acetate buffer, mixed 1:1 with 4% aqueous lanthanum nitrate, pH 7.6. All alcohols for dehydration were made using 4% aqueous lanthanum nitrate in place of water. Embedding, sectioning, and staining for electron microscopy were routine procedures [1].

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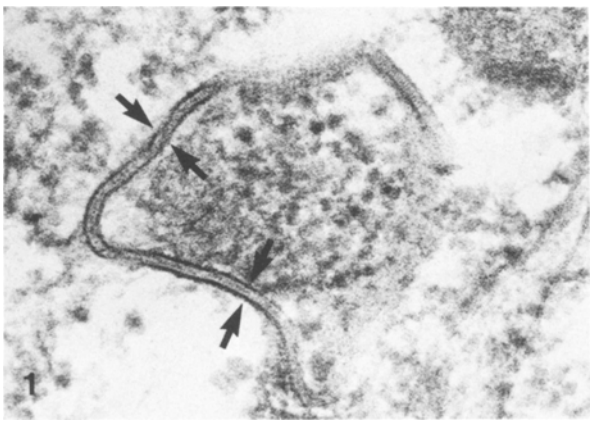

Fig. 1. Cell contact between adjacent osteoblasts appears as two dark lines (*arrows*) separated by a central density. With this routine preparation, the membranes appear to be fused. ×48,000

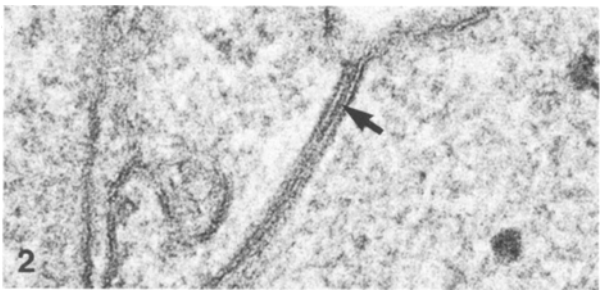

Fig. 2. Another routine preparation of the contact site between two osteoblasts. In this case, a space is seen (*arrow*) between the two sets of membranes. ×103,000

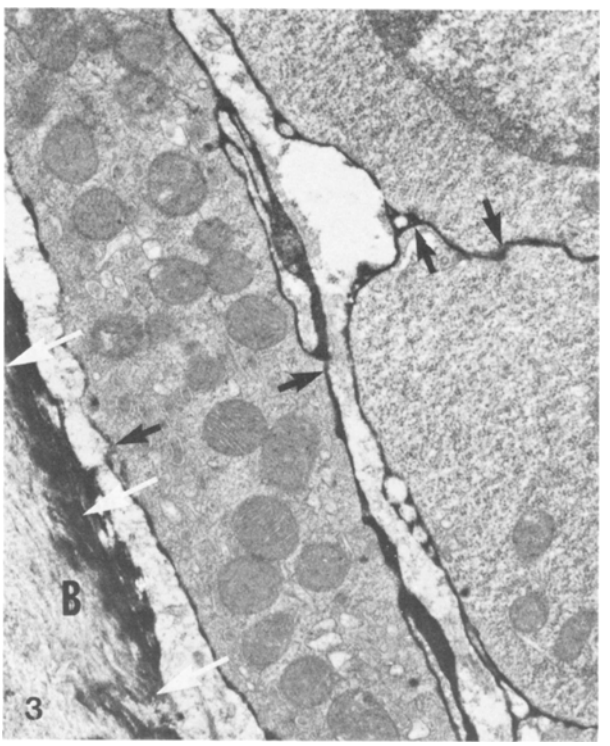

Fig. 3. Colloidal lanthanum fills the extracellular space between adjacent cells and is indicated here as an electron-dense line (*black arrows*). Some lanthanum (*white arrows*) fills the space between collagen fibers on the surface of mineralized bone (*B*). ×13,800

Results

The junctions between adjacent osteoblasts were visible with normal fixation, in the absence of lanthanum (Fig. 1). Depending on the plane of section, it often appeared that the outer adjacent membrane leaflets were fused. However, when the section plane was perpendicular to the membranes, a 2 nm gap could be seen between the opposing leaflets (Fig. 2).

Colloidal lanthanum infiltrated the extracellular space between bone and marrow cells (Fig. 3) and eventually penetrated the space between bone cells and the underlying matrix. Because the lanthanum was not chemically bound but only physically trapped between cells, the lanthanum could be lost during tissue processing. We found that the addition of lanthanum to osmium and alcohols helped to preserve the original deposits.

Lanthanum delineated the gap junction structure as found between adjacent osteoblasts and osteoblasts and osteocytes (Fig. 4). The lanthanum-filled spaces were seen to narrow down to a multilayered structure (Figs. 5 and 6). The layers of this structure consisted of the inner leaflets of opposing cell membranes, seen as single dense lines, adjacent to a lanthanum-free space. In the middle of this space was a dense lanthanum line 4–6 nm wide.

When lanthanum-containing junctions were sectioned at a slight tangent to the plane of cell membranes, a striated pattern appeared along the lanthanum line (Fig. 7). These striations resulted from lanthanum filling in the spaces between the bridging components of the gap region. These bridging structures were seen in negative image as a lanthanum-free space which crossed from one cell membrane to the other and at right angles to the lanthanum-filled gap.

When the plane of section occurred in the plane of the junction, or the same plane as the cell membrane face, a packing of polygonal-shaped structures could be seen (Fig. 8). In this case, the lanthanum outlined the polygonal subunits which crossed between cells to form the intercellular junction. The average center-to-center distance between subunits was 9 nm.

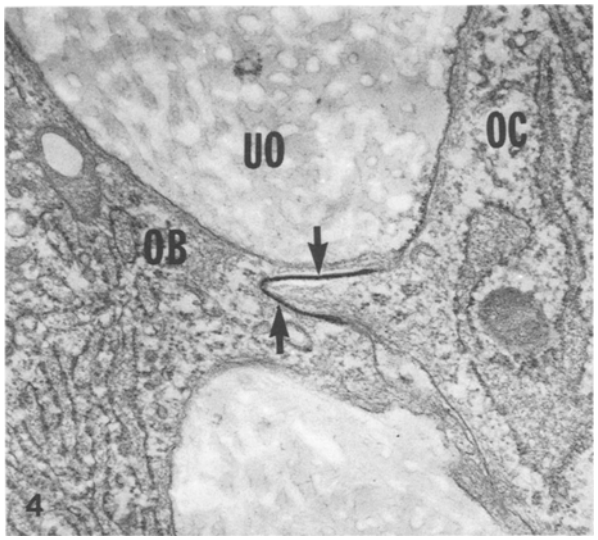

Fig. 4. A contact site between an osteoblast (*OB*) and an osteocyte (*OC*) is filled with lanthanum. The electron-dense line (*arrows*) represents a gap junction between the cytoplasmic processes arising from each cell type. Unmineralized osteoid (*UO*) surrounds the processes. ×24,000

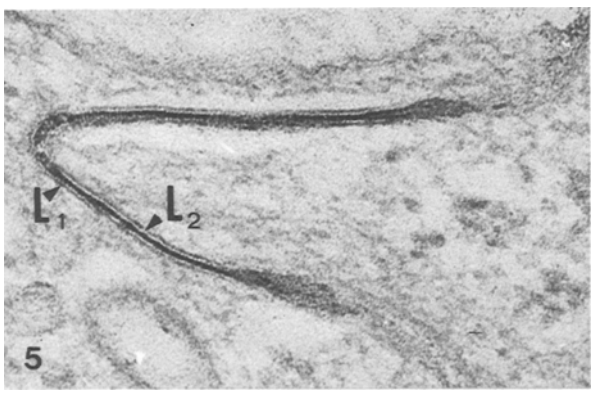

Fig. 5. An enlargement of the gap junction shown in Fig. 4. The inner leaflet of the cell membrane of each cell (L_1 and L_2) is seen as a line that is less dense than the central lanthanum line. ×109,000

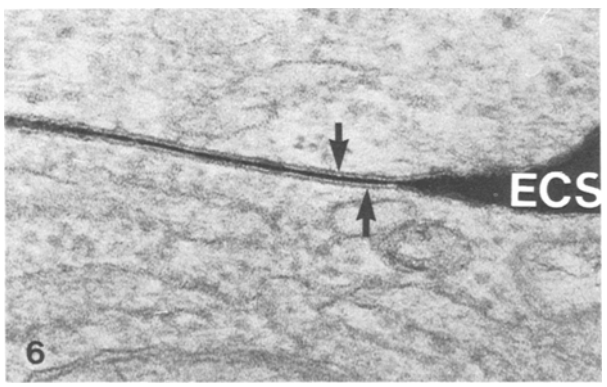

Fig. 6. As lanthanum penetrates the extracellular space (*ECS*) between two cells, a multilayered structure is seen, which consists of the two inner leaflets (*arrows*) of each cell membrane, a clear space, and a 4–5 nm wide lanthanum line. ×86,000

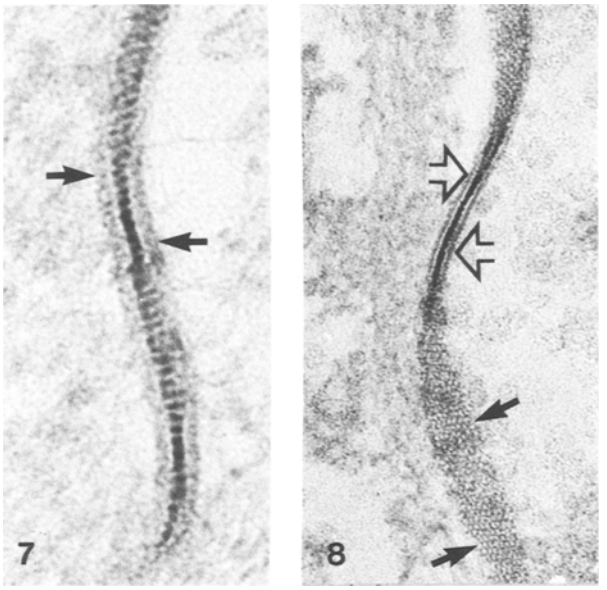

Fig. 7. At higher magnification, the contact site shows striations across the lanthanum line. These striations run from each inner leaflet (*arrows*) of the two cell membranes which are in contact. The striations are caused by "cross-bridging" structures within the contact site and which exclude lanthanum. The "cross-bridging" structures are spaced 9–10 nm apart. ×150,000

Fig. 8. The contact point between two osteoblasts shows the multilayered structure (*open arrows*) resulting from lanthanum penetration. On either side of this region, the cell membranes are sectioned parallel to the plane of the membrane surface, resulting in the appearance of polygonal structures (*solid arrows*). The cross-bridging structures form the central core of these polygons and exclude the lanthanum stain. ×80,000

These junctions with their intercellular connections were found between adjacent osteoblasts and between osteoblasts and osteocytes. We found no gap junctions between these mononuclear cells and osteoclasts. We also found no definite lanthanum-filled gap between adjacent osteocyte processes, but this was likely due to the technical difficulty of getting colloidal lanthanum to penetrate the deeper layers of compact bone during fixation.

Discussion

The morphological description of junctions between osteoblasts, obsteoblasts and osteocytes, and between osteocytes is documented by several investigators [1–5]. The resulting morphological descriptions of fused outer membranes and the use of the term "tight" or "occluding" junction suggest a mechanical function for these structures, although some of these studies speculate that the junctions may also be important for intercellular communica-

tion [2, 4, 5]. Our previous report [1] indicates that these junctions do not deter ions or small foreign proteins from moving around bone cells and rapidly penetrating the deep layers of compact bone. Therefore we suggest that these contact points between cells should not be considered as "tight" or "occluding" junctions since they do not meet the morphological or physiological criteria currently used to define these structures [6].

The study of cell membrane junctions in nonexcitable tissues [6–10] has led to the recognition that the gap junction is important for intercellular communication. The gap junction may provide a means for electrical coupling between cells, may be responsible for establishing contact inhibition between cells, and may permit ion or small protein transport between coupled cells [7–10]. Such a transport process is demonstrated by Jeansonne et al. [11] in bone cells as fluorescein moves through many osteoblasts after initially being injected into one cell. Molecules up to 1200 daltons molecular weight can pass intercellularly through the gap junction [7, 8].

The gap junction between bone cells, as described in this report, is identical to that shown for other tissues [6, 8–10, 12]. The lanthanum evidently penetrates the outer leaflets of the opposing cell membranes, so that the 2 nm gap which is present in routine morphology becomes a 5 nm wide lanthanum line [12]. It is interesting to note that the inner leaflets of the opposing cell membranes are not permeable to lanthanum. The infiltration of lanthanum and its physical trapping between the subunits of the gap result in polygonal structures being visible within the gap. The intercellular connections that cross between adjacent cells are also visible by this method.

The presence of gap junctions between bone cells may be of fundamental importance to many bone cell functions. Although ions and proteins can move through the extracellular pathways between bone cells and matrix [1], the intercellular pathways may play an important role by regulating cellular activity over a given area or within a given volume of bone. Thus the concentric lamellar pattern of bone in Haversian systems, the formation of bone at trabecular surfaces, and even the quiescent nature of periosteal surfaces of adult bone may all be influenced by the presence of this intercellular system of communication.

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