Membrane junctions on odontoblasts
A freeze-fracture study

ANDERS KÖLING, HELGE RASK-ANDERSEN & DAN BAGGER-SJÖBÄCK

Department of Otolaryngology, University Hospital, Uppsala, Sweden and Department of Otolaryngology, Karolinska Hospital, Stockholm, Sweden.

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The morphology of the odontoblastic cell layer in the human dental pulp was analysed using freeze-fracturing technique. Numerous intercellular junctions were found between individual odontoblasts as well as between odontoblasts and subodontoblastic cells (Höhl's cells). These junctions were of the gap junction type. Tight junctions were not observed.

Key-words: Freeze-fracture; dental pulp; intercellular junctions; ultrastructure

Anders Köling, Department of Otolaryngology, University Hospital, S–750 14 Uppsala, Sweden

Morphological studies of the odontoblastic layer have revealed different types of intercellular junctions. Areas of close apposition between cell membranes of adjacent cells have been described and classified as possible tight junctions (4, 6). It has been suggested (2, 10) that some of these membrane junctions might represent small gap junctions rather than the occludens type, since measurements of their thickness would put them in this category, according to McNutt & Weinstein (11).

The present paper reports the results of an investigation on the junctional morphology of the human odontoblastic layer, using freeze-fracturing. This technique offers unique possibilities of studying cell membranes and was used in order to try to establish the true nature of these junctions.

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MATERIAL AND METHODS

20 human molars and premolars extracted for orthodontic reasons from healthy individuals aged between 13 and 16 years were studied. The teeth were immediately placed in a solution containing 2.5 % v/v glutaraldehyde and 1 % v/v formaldehyde in 0.1 M of Sörensen's phosphate buffer at pH 7.2–7.4. Within 3 minutes the teeth were split in a screw-vise with cutting edges. Immersion fixation was performed immediately with the solution mentioned. After one hour the pulpal tissue was dissected away from the dentin and cut in small pieces under a dissection microscope. These pieces remained in fixative for at least 48 hours. After rinsing with buffer the specimens were transferred into a solution of 25 % buffered glycerol for 30 minutes and then

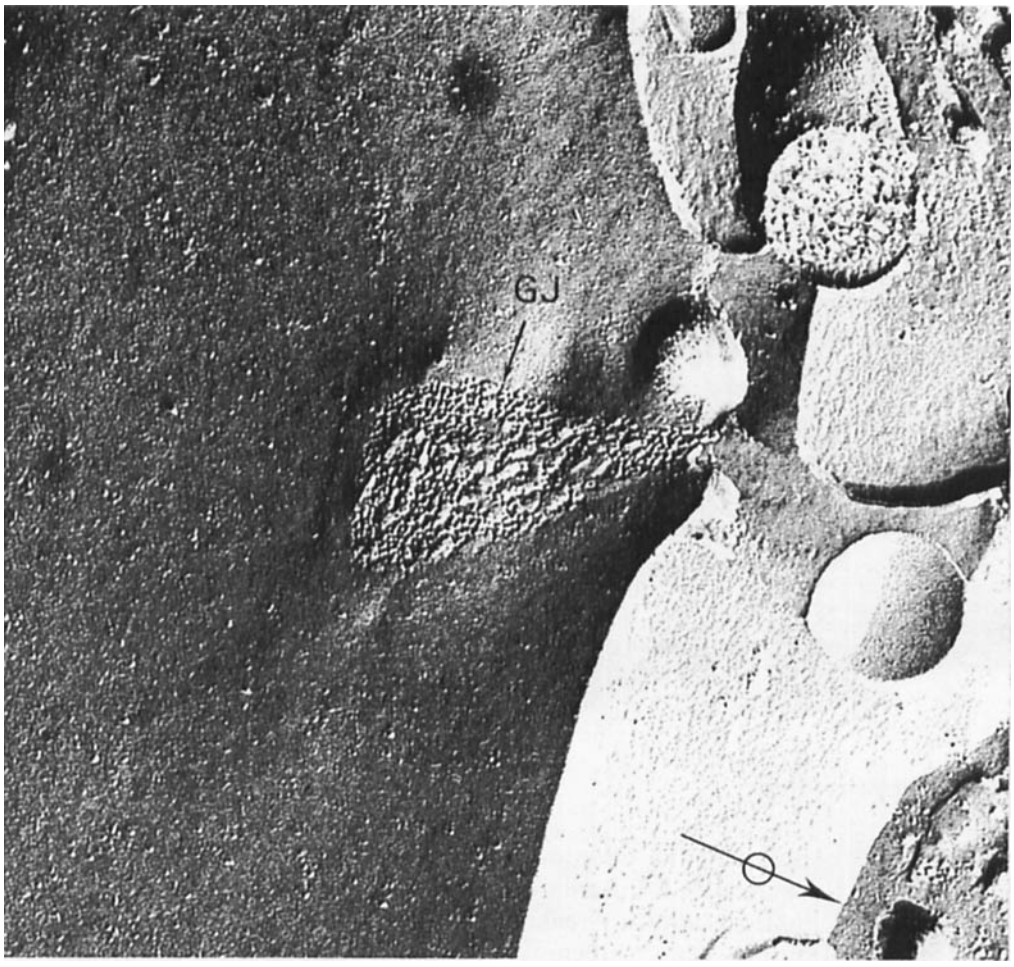

Fig. 1. Freeze-fracture replica of a human odontoblast exposing a gap junctional (GJ) area on the exoplasmic face. The aggregate of small membrane particles forms the pear-shaped junctional region. (Arrow indicates direction of platinum-shadowing.) 71 500 X.

mounted on goldcup specimen holders. The specimens were subsequently frozen in nitrogen slush and fractured in a BAF 360 M (Balzers Ag, Lichtenstein) freeze-etching apparatus with double replica technique (12). The fractured surfaces were replicated with evaporated platinum and carbon according to conventional methods. The biologic tissue was then digested in NaHCl and the resultant replicas were mounted on copper meshgrids and examined in a JEOL-100 B electron microscope at 80 kV.

OBSERVATIONS

Freeze-fracture replicas of the human odontoblasts showed numerous macular aggregates of membrane particles, representing gap junctions. These were of various size and shape when viewed en face (Fig. 1). Oblique fracturing showed the intercellular space between the cells to be reduced to 2 nm (Fig. 2) and, when cross-fractured, a similar reduction of the intercellular space could be seen (Fig. 3). The opposed membranes appeared slightly granular.

The membranous specificities of gap

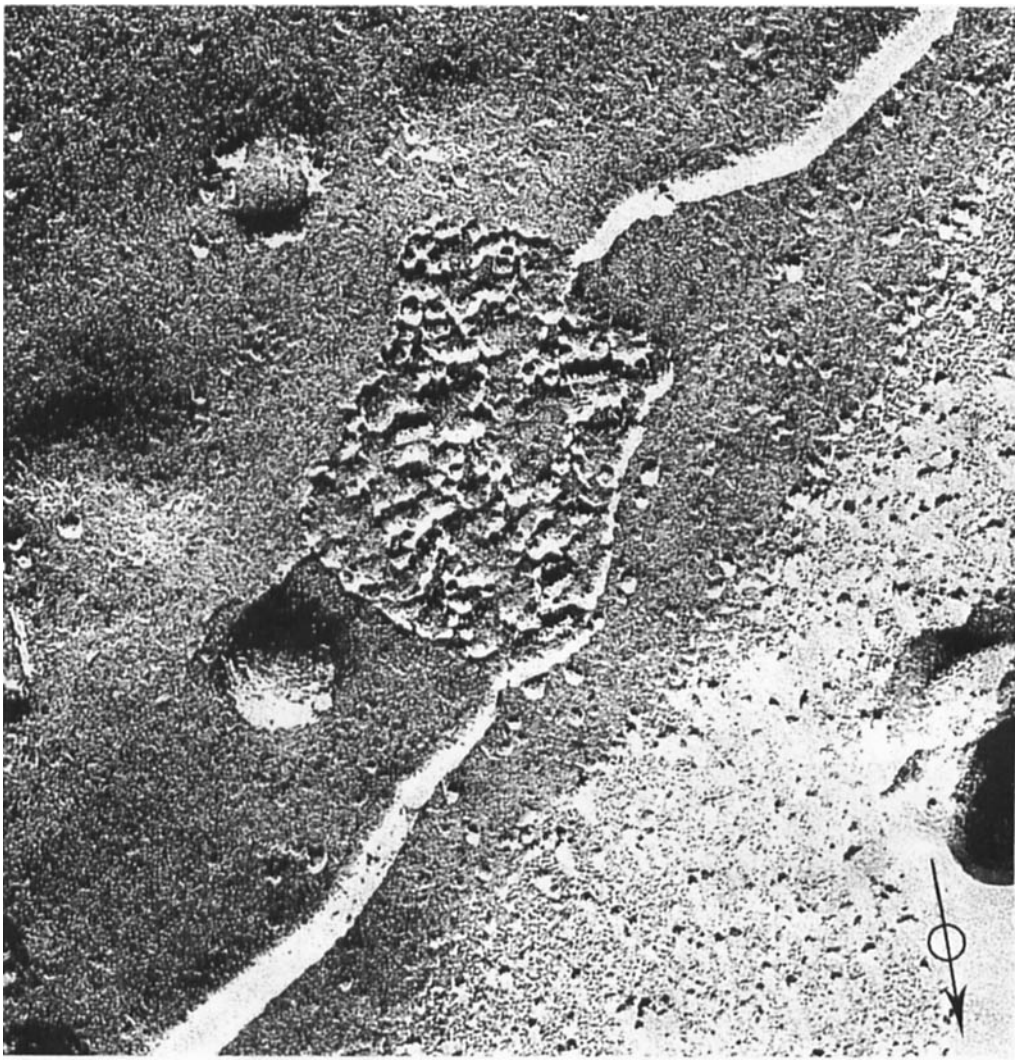

Fig. 2. Gap junctional area between two odontoblasts. The intercellular space narrows characteristically to the level of the junctional area. 195 000 X.

junctions were most easily identified between individual odontoblasts. They were also found in great numbers in the contact area between the sub-odontoblastic cells (Höhl's cells) and the odontoblasts. Slender processes of Höhl's cells formed bouton-like, cellular contacts on the odontoblasts, which in the freeze-fractured material were of gap junction type.

Despite careful examintion, no tight junctions were disclosed. There were no areas of fused membranes with the ridges or grooves which are characteristic of tight junctional complexes. However, near the predentin surface the distance between the odontoblasts was reduced to about 8–10 nm, and on corresponding thin-sectioned material the areas could be classified as zonulae adherentes.

DISCUSSION

The odontoblast layer is a highly

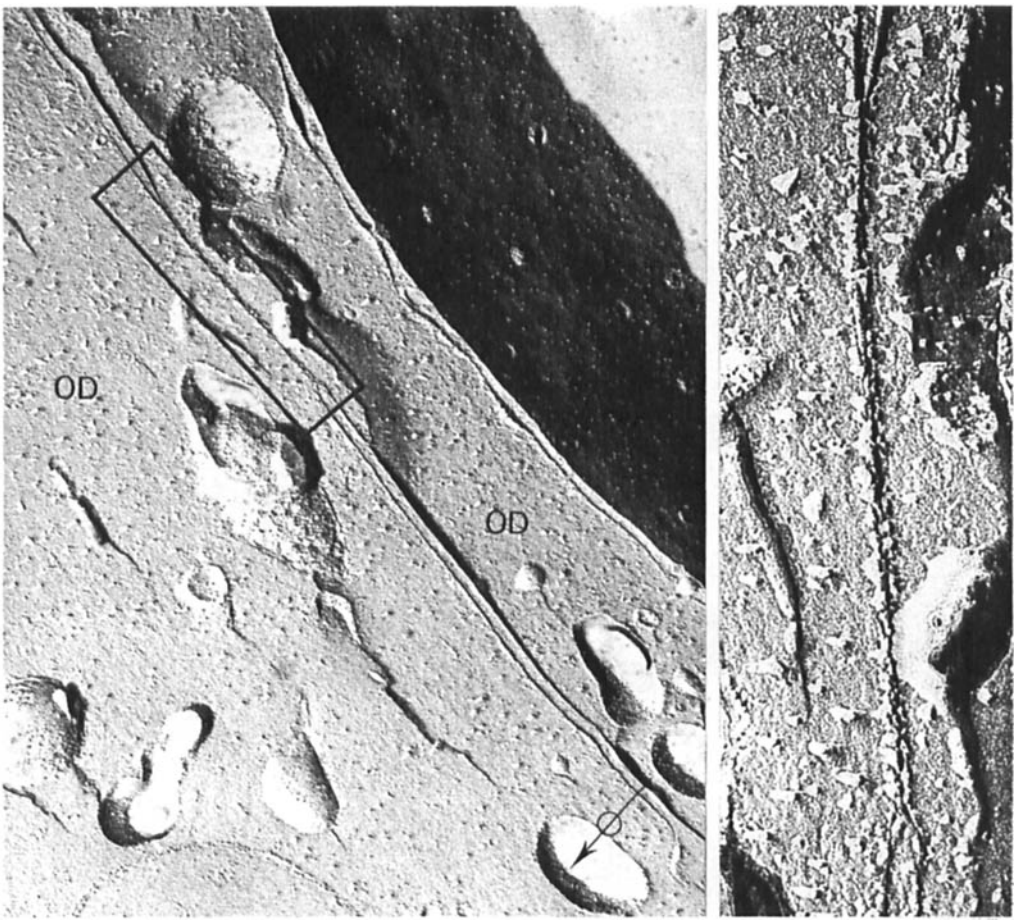

Fig. 3. Freeze-fracture replica of the human odontoblastic cell layer. Two odontoblasts (OD) are split exposing their interior cytoplasmic structures. A typical gap junctional area is seen and is shown in higher magnification to the right. The cells are separated by a 40 nm wide intercellular space which is reduced at the site of junctional communication. 32 000 X, 103 000 X.

specialized structure with different important functions. As a protective, marginal layer, it enclaves the dental pulp and is responsible for the formation and nutrition of the dentin.

The type and relative occurrence of intercellular junctions may reflect important functional properties of the cells or groups of cells. New staining methods and the freeze-fracturing technique have increased our knowledge of the components of the intercellular, junctional complexes.

The zonula occludentes or tight junctions form a closing belt around the apical area of the individual cells in various epithelia. The outermost layers of the cellular membranes between adjoining cells fuse and obliterate the intercellular space. Earlier morphological studies of the dental pulp in various species have revealed the presence of junctions or plasma membrane specilizations between odontoblasts. These cells are not of epithelial but of mesodermal origin. The junctions have been characterized as possible tight junctions (4, 6). The introduction of new

staining techniques (e.g. lanthanum) and the freeze-fracture method made it possible to separate the gap junctions from the tight junctional group to which they were erroneously believed to belong. Lanthanum in colloidal form could penetrate along the intercellular cleft into the gap junctional area revealing a hexagonal array of particles between the cells. The fundamental difference between gap and tight junctions was further elucidated with freeze-fracture where the latter junctions were exposed as a pattern of thin membranous ridges or grooves. In our study, we could not detect any junction which could be classified as tight. However, it should be remembered that this study was performed on split teeth, in which the cleavage of the hard tissue sometimes resulted in traumatization of the most peripheral part of the odontoblastic cell layer. On the other hand, we could identify what have earlier been classified as tight junctions as being in fact true gap junctions. Besides, it is hard to understand how nerve fibres and collagen fibres would be able to pass between the odontoblasts if tight junctions were present.

The gap junction has been shown to represent an area of low electrical resistance, allowing the movement of ions (11), metabolites and small, molecular-size substances from cell to cell (7). It is believed that these cell-to-cell communications play a role for the ability of the cells to act in concert. Our study showed that the gap junctions were confined not only to the area between odontoblasts but also to that between these cells and Höhl's cells. It was striking how often these membrane specializations occurred. Presumably they reflect important physiological processes. Gotjamanos (9) suggested that Höhl's cells may be important for the metabolic assistance to the odontoblasts. These sub-odontoblastic cells may provide a pathway for the nutritional supply of the odontoblasts. On thin-sections, numerous cellular contacts were observed between odontoblasts and Höhl's cells and frequently these contacts were specialized into intercellular junctions, which on freeze-fracturing replicas are seen to represent gap junctions.

Zonulae adherentes, which were found near the predentinal surface between the odontoblasts, possibly assist in keeping the cellular layer patent, since they are thought to serve as insertions for microfilaments of the terminal web and to represent an intercellular attachment device (3).

Finally, another topic that may be discussed is the possible role of the gap junctions in the neurosensory supply of the pulp. There is still disagreement about the localization of the peripheral nerve terminals of the dental pulp. Baume (1) states that, in spite of the incontroversial histological evidence, the subject of dentin sensitivity still remains a matter of discussion. Contacts between nerve endings and odontoblasts have been identified (4). Further studies of the relationship between the terminal axons and odontoblasts are needed in order to clarify the generation of dentinal pain impulses. The freeze-fracture method should be an ideal way of studying such communications. Such a study is under progress in Uppsala.

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