

# Intercellular junctions between odontoblasts as the transmitters of low charge electric current

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## Abstract

The aim of the work was to demonstrate structures responsible for the conduction of pain impulses with regard to tight junctions after transmission of small charges of electric current. For the experiment, freshly extracted (for orthodontic reasons) teeth were used. Immediately after extraction, low charges of electric current were passed through the teeth and the teeth were fixed. Teeth pulps were prepared for TEM studies. The obtained results were compared to those in a control group. In electron microscopic pictures, intercellular junctions are visible irrespective of the value of the current applied on the approximal surfaces of the cells (between the odontoblasts or between the odontoblasts and the neural fibres) as short callosities of plasmolemma.

**Key words:** odontoblasts, belt desmosom, alternating current, TEM, active transport of water.

## Introduction

Apart from many other functions, the odontoblasts are involved in the process of perception and conduction of pain stimuli from the tooth during stomatological procedures. A precise recognition of the structures connecting the enamel through the dentine with the dental pulp, is a major aspect of dental science. The knowledge of these structures would give us a better understanding of many physiological processes.

In 1968, Frank, for the first time, described nerve endings that formed junctions with odontoblast processes in the first zone of

dentinal tubules [1]. The studies of dental pulp, predentin and dentin in electron microscope, carried out by this author and next by other researchers [2, 3], have resulted in a conclusion that there are numerous junctions between belt desmosome (zonula adherens), tight junctions (zonula occludens) and gap junctions (nexus) [4]. However, according to Sasaki, it is perhaps more accurate to use the terms fascia adherens and fascia occludens to describe the components of the modified distal junctional complex [5, 6, 7]. This does not change the fact that such junctions enable the, so-called, junctional transfer - a free flow of ions between cells, so they play a function of electric synapses [8, 9]. The presence of these junctions is also suggested by SEM images [10]. Structures, resembling synapses, which occur between the odontoblast processes and neurofibrils have also been reported; those were callosities of plasmolemma near which synaptic vesicles of 50-60 nm in diameter and agglomerations of mitochondria [1] could sometimes be observed [1]. Physiological and cytophysiological features of odontoblasts may suggest that they can be included to the class of the, so-called, paraneurons [11]. Three mechanisms of active transportation are known: 1) group translocation (in bacterial cells), 2) primary active transport and 3) secondary active transport. In the cells of dental pulp, primary and secondary active transports play the role. In the typical cell ion gradients, transversally to the cell membrane, high concentrations of Na<sup>+</sup> and Cl<sup>-</sup> get into extracellular space and high concentration of K<sup>+</sup> ions get inside the cell. Ion concentrations balance the osmotic pressure and prevent an influx of water into the cell. The aim of this study was to present structures responsible for the transmission of sensory and pain reflexes, taking into account zonula adherens, after transmitting through them small electric charges.

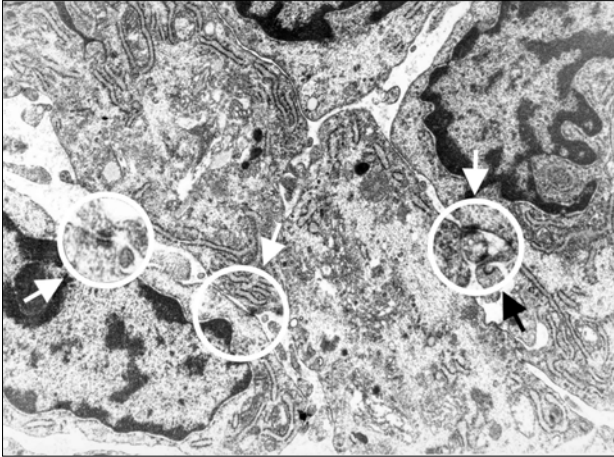
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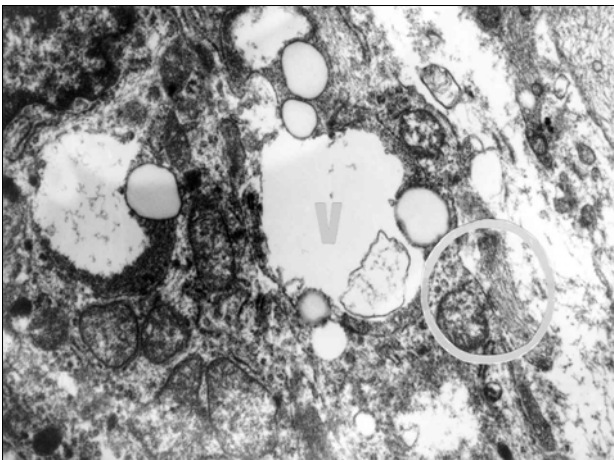
## Materials and methods

The studies were carried out on non-carious teeth, extracted for orthodontic reasons. The teeth were extracted with the use of local anaesthesia. Immediately after extraction, small charges

*Figure 1.* Dental pulp odontoblasts of the premolar tooth (-4), treated with electric current of 50  $\mu$ A during 120 sec. Zonulae adherens - of desmosome type, surrounded with circles (white arrows). Black arrow shows the gap junction inside the circle of hemidesmosome (nexus) type. The first circle shows desmosome in the state of repose; the second and the third circles - an active stage. Magnification 30000 x.



*Figure 2.* Zonulae adherens (circle) between the axon and odontoblast in active stage, near the vacuole (V). Dental pulp of the premolar tooth (+4), treated with the electric current of 80  $\mu$ A during 120 sec. Magnification 25000 x.



(15  $\mu$ A, 50  $\mu$ A, 60  $\mu$ A, 80  $\mu$ A for 120 s) of alternating current were transferred through the teeth and then, the teeth were fixed in 2% glutardialdehyde in cacodylate buffer. The teeth were cut and their pulps were fixed in 2% glutardialdehyde in cacodylate buffer and then, in 1% osmium tetroxide. After that, they were dehydrated and embedded in epoxy resin (EPON 812 with DDSA and MNA) for electron microscopic studies. Hemithin sections were prepared with the use of a Tesla BS 480 ultramicrotome and stained with buffered 1% toluidine blue. Ultra thin sections were cut in an MTI ultramicrotome and contrasted with uranyl acetate and lead citrate. They were investigated and photographed in a JEOL electron microscope. The results of the studies were compared with the image of dental pulp tissue through which no electric current had been transmitted and in which, no changes were revealed by the hard tissue. The pulp was also fixed immediately after the teeth extraction, using the method described above.

## Results and discussion

In the specimens, through which current from 15 mA to 60 mA passed, belt desmosomes (zonula adherens) and gap junctions were not damaged. These juncturae adherentes are a space of 15-25 nm in width between the adjacent membranes, which contains electron dense material (Fig. 1). On the side of cytoplasm, there is a condensation of hyaloplasm and actin microfilaments. At higher magnifications, different activity stages (passive and active) are observed [12, 13]. The anatomical shape of belt desmosome reminds condensing lenses, what is of great importance in water and ions transportation; electric current (depending on the kind and value) intensifies these processes. As far as the alternating current is concerned, in most specimens, through which current of 80  $\mu$ A was passed, a higher number of small vacuoles appeared at unchanged belt desmosome (zonula adherens) (Fig. 2). It is worth noting that, in analogical studies with the application of alternating current of similar parameters, fine vacuoles were not visible in a light microscope [14].

In the specimens, prepared for electron microscopy, on the side surfaces of cells (odontoblast-odontoblast, odontoblast - neuron, odontoblast - neutrophil) indirect junctions were observed in the form of callosities of plasmolemma. These junctions enable the, so-called, junctional transfer - a free flow of ions between the cells, thus playing the function of electric synapses. The results of the studies confirm the previously described junctions, observed in SEM.

It can be presumed that, depending on current intensity, a faster accumulation of vacuoles, that appear near the odontoblast nucleus, could induce detachment of a part of cell cytoplasm near zonula adherens (juncturae adherentes), observed in SEM (that was probably related to the technical process of routine specimen preparation), described by H. Alwas-Danowska [10].

In the dental pulp cells of the experimental group, the cellular membranes and tight junctions were intact, but in the control group the picture of the junctions was indistinct, what could be connected with the water penetration. Because of their anatomical shapes, zonula adhaerens (resembling condensing lenses) in the odontoblasts have a possibility of water intensification and ion transportation processes and the passing electric current of low charges could change them.

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