Distribution and Organization of Odontoblast Processes in Human Dentin

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Summary. The distribution and organization of odontoblast processes in young human dentin was examined with a scanning electron microscope. By applying the HCl-collagenase method, the extracellular matrix of dentin was almost completely removed, thereby exposing the odontoblast processes and their branches to direct observation. The odontoblast processes are located close to the dentinoenamel junction. In the middle and outer zones of dentin the processes bear numerous branches. Some of these appeared to bridge the space between the processes and connect them.

The present scanning electron microscope study was undertaken in order to gain more detailed information on the distribution of odontoblast processes in calcified dentin.

MATERIAL AND METHOD

Extracted intact premolars from children aged 10 to 12 years were used in the present study. Immediately after extraction the teeth were immersed in liquid nitrogen where they usually cracked by themselves along the buccolingual plane. Cracked tooth blocks
Fig. 1. SEM of the pulpodental border zone. D the inner zone of dentin, PD predentin, OL odontoblast layer. ×800

Fig. 2. SEM of the middle zone of coronal dentin. OP odontoblast processes. ×2,500
were immediately fixed in a 0.1 M cacodylate buffered mixture of 2.0% formaldehyde and 2.5% glutaraldehyde (pH 7.2) at 4°C for 24 hrs. The specimens were subsequently immersed in 5 N HCl for 1–2 hrs to remove the enamel and expose the outer surface of the dentin. Following washing in distilled water, the outer surface of dentin was covered with epoxy resin (Araldite: Ciba-Geigy), which served to mark the position of the dentinoenamel junction after the next digestive treatment for removing the extracellular dentin materials. The specimens were then treated with the HCl-collagenase method (Evan et al., 1976) and again thoroughly washed in distilled water. They were postfixed in a 1% osmium tetroxide solution, dehydrated in a graded series of acetone, substituted with isoamyl acetate, dried in a critical point drier and gold-coated in an ion sputter coater. The specimens were observed with a Hitachi SSM-2 scanning electron microscope at an accelerating voltage of 20 kV.

RESULTS

Although treatment with the HCl-collagenase method was not sufficient for the predentin, cementum or pulp, most of the extracellular matrix of dentin was removed. Consequently, an odontoblast process extending from its cell body could be directly observed without the obstacle of the dentin matrix (Fig. 1). For the convenience of description, the dentin is divided into the following three zones: inner zone, middle zone and outer zone. The outermost layer of the outer zone, which is adjacent to the dentinoenamel or dentinocementum junction is called the superficial zone.

In the coronal dentin, the odontoblast processes pass through the predentin, the matrix of which extends upward to the inner zone of the dentin proper. The processes were observed to be tubular structures with a diameter of 1–2 μm (Fig. 2). Although branches were also seen, they were
rare in the inner zone (Fig. 4). In the middle zone, the diameter of the odontoblast processes decreased slightly and the spaces between them appeared wider. Numerous branches were present in this zone (Fig. 2). These branches projected mainly toward adjacent processes. The tips of some of them made contact with another process, giving the branches the appearance of cross-bridges connecting two neighboring processes (Fig. 3). Even in the outer zone many odontoblast processes were recognizable, but here their diameter decreased further to about 0.2-1.0 μm. The pattern of branching in the outer zone was similar to that seen in the middle zone, although branching was more extensive in the former (Fig. 2, 3). In the superficial zone the odontoblast processes gradually become thinner and terminate freely without any specialized structures very close to the dentinoenamel junction (indicated by the epoxy resin wall, Fig. 5). On the surface of the epoxy resin wall facing the outer surface of dentin, cellular fragments possibly corresponding to the distal ends of odontoblast processes were occasionally seen (Fig. 7). This suggests that some processes may reach the dentinoenamel junction and even penetrate it for a very short distance.

In the root dentin, odontoblast processes also extended into the superficial zone (Fig. 8). As in the coronal dentin, numerous branches were present, but in this case the inner zone possessed a larger number.

Fig. 5. SEM of the superficial zone. DEJ dentinoenamel junction which is indicated by epoxy resin wall. \( \times 1,800 \)

Fig. 6. SEM of the outer zone. \( \times 1,500 \)
DISCUSSION

As shown in the present study, the action of the HCl-collagenase method, which has recently been employed for removing the extracellular matrix (UEHARA and SUYAMA, 1978; NAGATO, 1978), was so effective that the extracellular dentin matrix was completely dissolved. The present study was, therefore, able to reveal that the exposed odontoblast processes extended throughout the thickness of the dentin, in this case young, normal, premolar dentin. This also suggests that the odontoblast processes may function as an intermediator of stimuli in the middle and outer zones of dentin which are non-innervated (AVERY and RAPP, 1958; MATTHEWS and HOLLAND, 1975; GUNJI, 1982).

These observations do not support the opinions expressed by TSATSAS and FRANK (1972), GARANT (1972) and HOLLAND (1975, 1976). However, our findings cannot be easily compared with theirs. Both the difficulty in the histological fixation of the dentin and the differences in the age of the examined teeth must be considered before making a comparison, as there may be factors inherent in the material or in the methods which would result in a discrepancy between our findings and theirs.

Optimal fixation is a problem because the fixative can infiltrate dentin only very slowly. Even if teeth are intravascularly perfused with fixative, the infiltration is as slow as that during immersion fixation because dentin has no blood supply. Moreover, the odontoblast processes in the dentinal tubules are surrounded by a wall of calcified matrix so that it is very difficult to achieve good histological preservation by a standard fixation method. In

Fig. 7. A surface (Se) of the epoxy resin wall indicating the position of the dentinoenamel junction. OP odontoblast processes. × 8,000

Fig. 8. SEM of the outer zone of root dentin. DCJ dentinoenamel junction. × 400
the present study, however, the teeth were cracked in liquid nitrogen to expose their surfaces for observation immediately after extraction, and were then immersed in the fixative. Therefore, we can be confident that the observed surfaces were fixed almost immediately after extraction, and artifacts resulting from a delay in fixation are minimal in the material.

It is quite possible that the distribution of odontoblast processes in dentin may be different depending on the age of the examined teeth. This question, however, can only be discussed when more data on the distribution of odontoblast processes in teeth of various ages become available.

In the middle and outer zones of the coronal dentin, numerous branches of odontoblast processes were recognizable; they were rare in the inner zone, whereas in the root dentin many branches were observed along the total length of the odontoblast processes. This distribution of the branches is very similar to that of the lateral branches or canaliculi of dentinal tubules (Fujita, 1957). The branches of the odontoblast processes would also course through the canaliculi. In addition, the present study showed that a branch of odontoblast processes does contact with another process. This contact between the branches of adjacent odontoblast processes is presumed to be represented by a specialized junction. This possibility deserves confirmation as the contact seems able to survive the severe HC1-collagenase treatment and be well preserved.

Odontoblasts and osteocytes have some characteristics in common, developmentally as well as functionally and morphologically. It is known that the cytoplasmic processes of osteocytes contact each other by tight junctions (Bloom and Fawcett, 1975). It is then reasonable to propose the possibility that the branches of odontoblast processes might also contact adjoining processes by tight junctions, anchoring the cell processes to each other.

REFERENCES
