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Original Article

Relationship between Large Tubules and Dentin Caries in Human Deciduous Tooth

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Abstract

The purpose of this study was to elucidate the relationship between large tubules and dentin caries by using human deciduous incisors that showed various levels of attrition but no macroscopical lesions resulting from caries. The teeth were cut longitudinally in the mesio-distal direction and the exposed surfaces observed with a high-resolution field emission scanning electron microscope. The inside of each large tubule showed dense collagen fibers running parallel to its long axis and small spherical bodies of aggregated crystals, but no marked attrition. In teeth where attrition had exposed dentin at the incisal edge, oral bacteria had infiltrated the large tubules. Furthermore, in teeth with advanced attrition, it was difficult to distinguish between the large tubules and the surrounding dentin matrix, and numerous bacteria were observed in both areas. These findings support the hypothesis that large tubules play a role in the pathway of caries formation in coronal dentin when incisal dentin is exposed by attrition. This suggests that early treatment of exposed dentin surfaces might be effective in preventing dental caries.

Key words: Large tubule—Deciduous tooth—Dentin caries—Attrition—SEM

Introduction

Several reports have been made on the large-diameter tubules which are found in coronal dentin[1–3,6,7,10,11,15,23]. We have already reported the distribution and internal structure of large tubules in human deciduous teeth where those specimens were clinically

caries-free and showed little attrition[2]. In another report, using optical microscopic observation of longitudinal sections of tooth, we found that these large tubules extended from the vicinity of the incisal edge of the dentino-enamel junction to near the secondary dentin or pulp cavity, running parallel to the dentinal tubules[1]. In cross section, they were found to be arranged linearly in the mesio-distal direction, and were only present in the labio-lingual central portion of the coronal dentin. It was clear that the incidence of large tubules was higher in deciduous teeth than in permanent teeth. Electron microscopic observation of the internal structure of large tubules has shown that they are filled with collagen fibers and aggregated crystals, but no odontoblast processes or other types of cell[1,3].

Although coronal dentin is covered by enamel in human intact teeth, incisal dentin is easily exposed by attrition. When large tubules are exposed to the oral cavity by advanced attrition, they may become portals for oral bacteria. This suggests that such bacterial invasion induces dental caries in dentin. Therefore, we examined the intratubular and peripheral structure of large tubules in human deciduous dentin with various levels of attrition by using a high-resolution field emission scanning electron microscope (FESEM) to elucidate the relationship between large tubules and dentin caries lesions.

Materials and Methods

1. Teeth preparation

We used 25 human deciduous incisors (3 maxillary central deciduous incisors; 4 maxillary lateral deciduous incisors; 8 mandibular central deciduous incisors; 10 mandibular lateral deciduous incisors) obtained from patients ranging in age from 5 to 8 years. The parents who provided these specimens agreed to the purpose of this study. The enamel of the teeth was clinically caries free, and the degree of root resorption was 0.5 to 0.75 of their original length. Level of attrition varied from teeth with attrition limited to the enamel to teeth with oval dentinal exposure. The teeth were extracted and immediately fixed in 4% paraformaldehyde solution for at least 1 week, after which they were washed in distilled water in order to remove the fixative. The mesial and distal surfaces of the teeth were ground down with a grindstone to expose the pulp cavity. The specimens were then frozen with liquid nitrogen and fractured longitudinally in the mesio-distal direction through the central portion of the incisal pulpal edge with a chisel (Fig. 1). This was to expose the large tubules, which are arranged linearly in the mesio-distal direction and are distributed only in the labio-lingual central portion of incisal dentin.

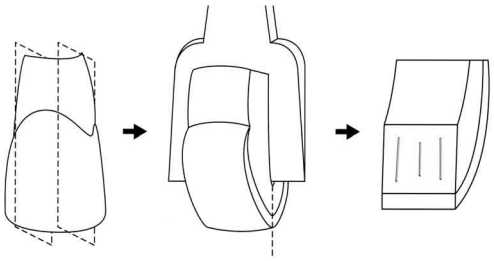

Fig. 1 Procedure for exposing large tubules

2. Scanning electron microscopy

The fractured specimens were given a 2-minute ultrasonic wash in distilled water 3 times, with a change of water each time. They were dehydrated through a graded ethanol series, immersed in t-butyl alcohol, frozen, and then dried in an Eiko ID-2. They were then gold-palladium coated using a cool sputter coater. The fractured surfaces were observed with the FESEM (JEOL JSM-6340F) operated at 15 kV.

Results

The large tubules, clearly observed as dark grooves, ran toward the pulp cavity from the vicinity of the dentino-enamel junction of the incisal edge. Attrition was minimal, that is, only part of the incisal dentin was exposed,

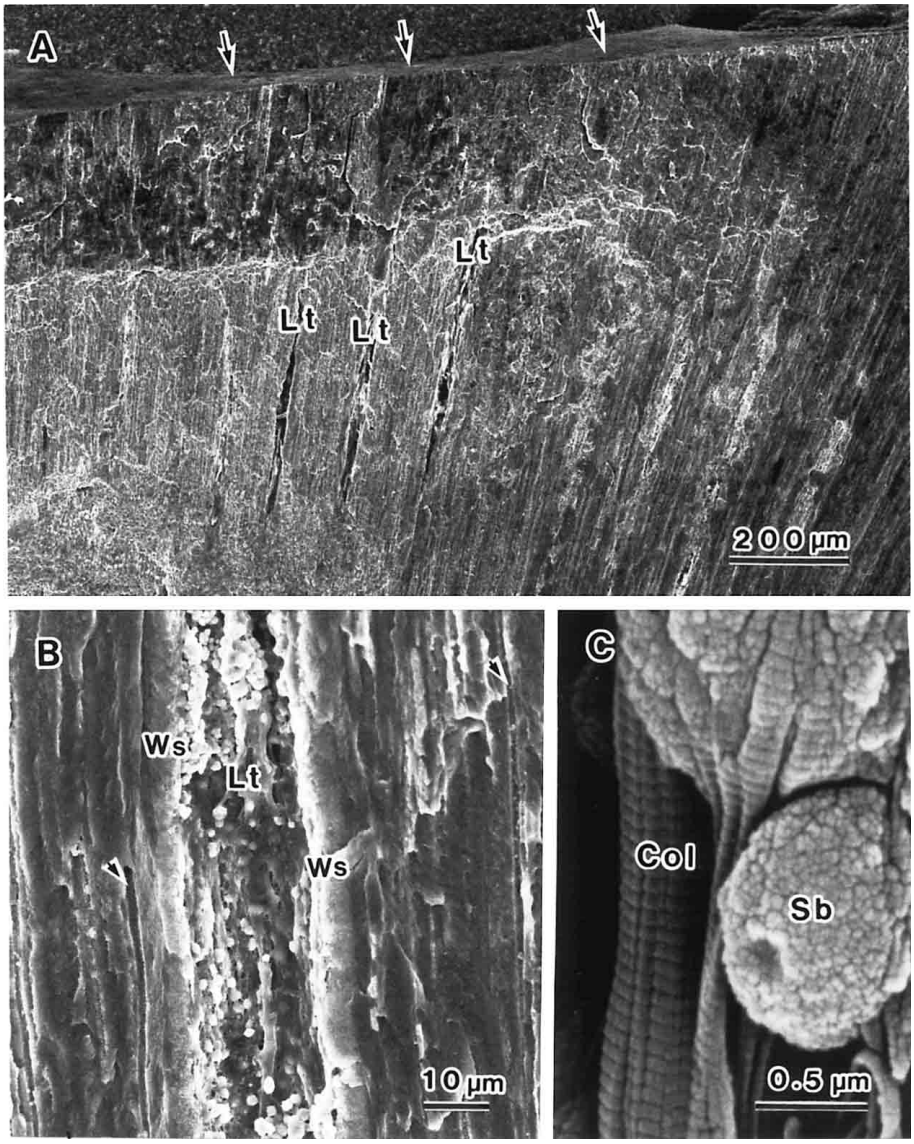

Fig. 2 FESEM photographs in case of slight attrition

(A) Low magnification of micrograph in case of slight attrition. Large tubules (Lt) are evident as grooves extending from near incisal edge (arrows) towards pulp cavity (below area shown in photograph).
(B) Large tubule (Lt) bigger in diameter than dentinal tubules (arrowheads); has glossy wall structure (Ws) similar to that in peritubular dentin of dentinal tubules. Interior of large tubule is composed of bundles of longitudinally oriented fibers and numerous spherical shaped bodies.
(C) Higher magnification of interior of large tubule. Longitudinally oriented collagen fibrils (Col) with periodical cross striations and spherical shaped bodies (Sb) made up of aggregates of crystals.

revealing island-like patches. The large tubules more or less converged in the vicinity of the pulp, running parallel to the dentinal tubules in the mesio-distally fractured dentin (Fig. 2A). Around the periphery of the large tubules, there was a glossy wall-like structure, similar to that of the peritubular dentin of general dentinal tubules. The interior of the large tubule had a diameter of approximately 20μm, and was largely composed of bundles of fibers run-

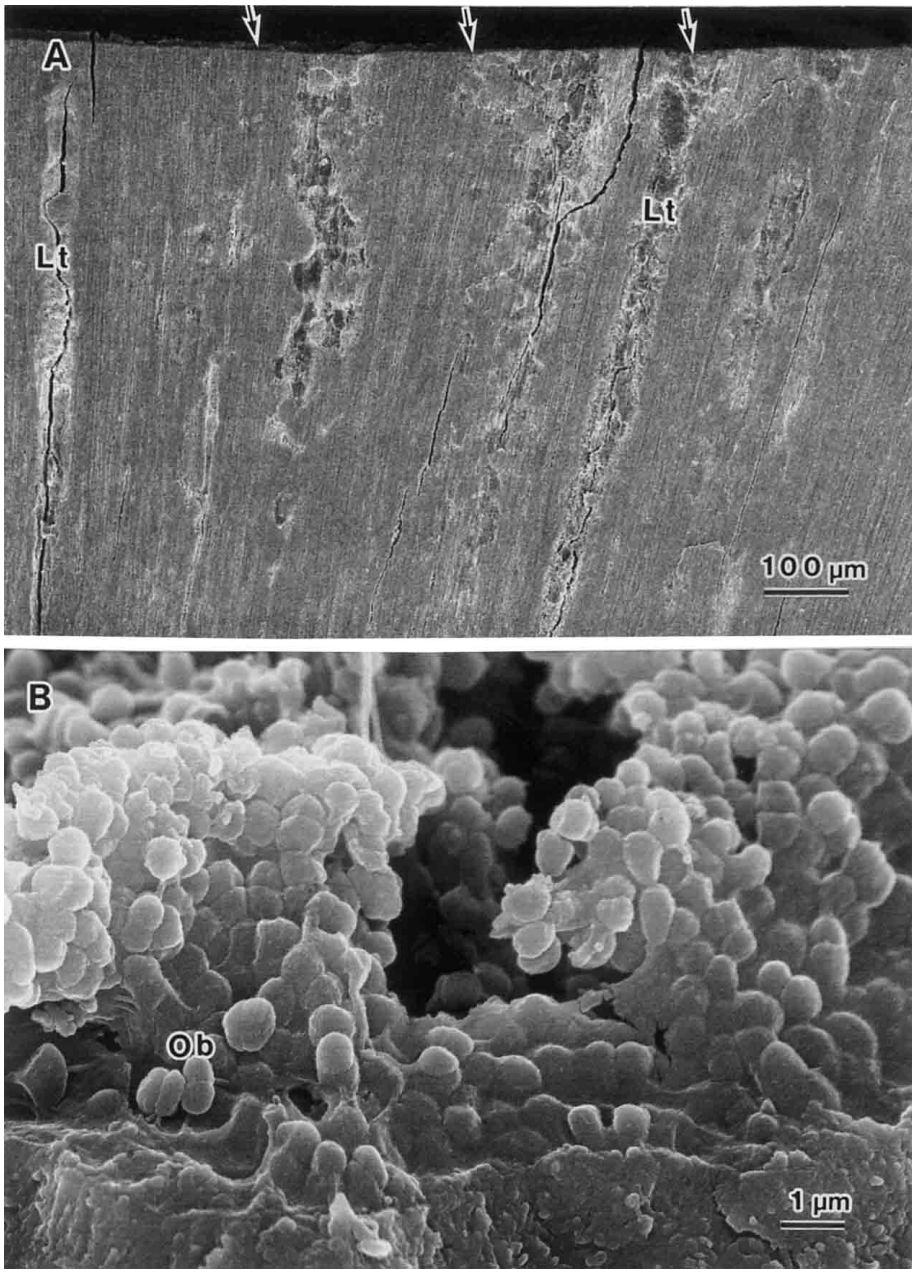

Fig. 3 FESEM photographs in case of advanced attrition
(A) Low magnification of micrograph. Large tubules (Lt) opening onto incisal dentin surface (arrows).
(B) Occlusal surface of dentin is covered by numerous oral bacteria (Ob).

ning longitudinally. Small spherical bodies were observed between these bundles of fibers (Fig. 2B). Enlargements of the interior of the large tubules further revealed longitudinally oriented fiber bundles. These bundles of fibers consisted of collagen fibrils. They were cross-striated in structure with a periodicity of approximately 60–70 nm, and ranged from 80–150 nm in diameter. Spherical bodies composed of aggregated crystals were also

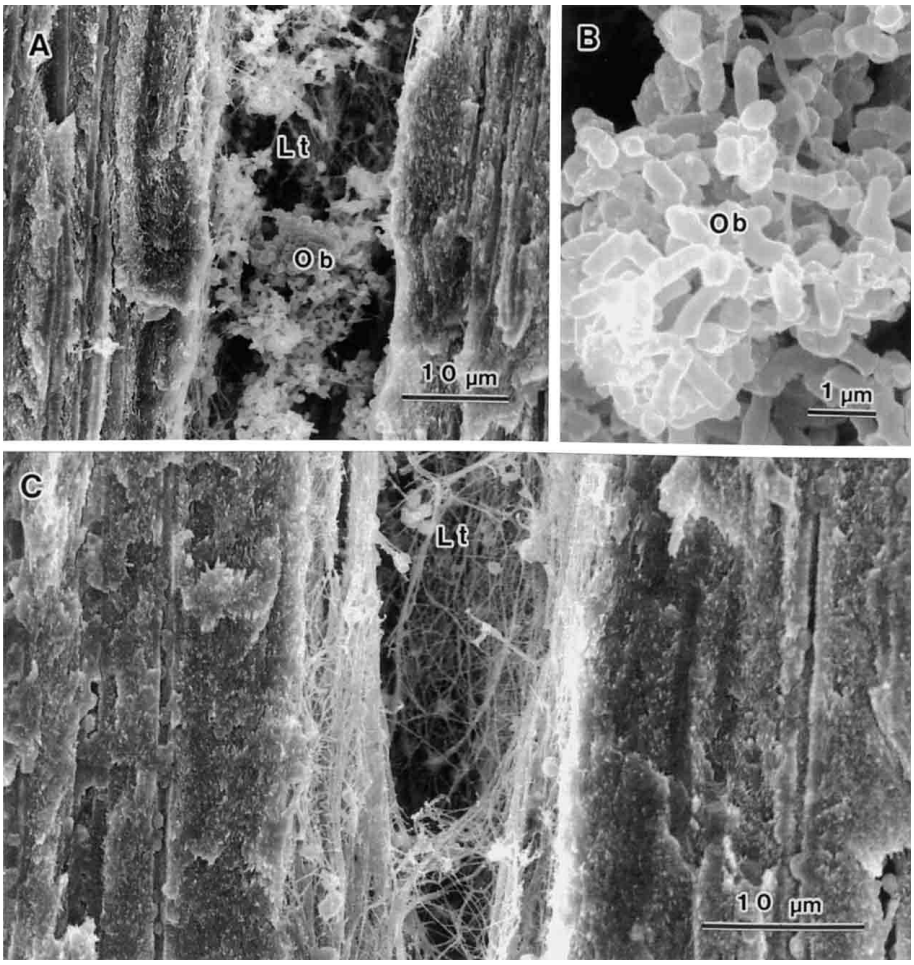

Fig. 4 FESEM photographs showing interior of large tubule in advanced attrition
(A) Oral bacteria (Ob) in large tubule (Lt) close to incisal openings. Few collagen fibril bundles and small spherical bodies, and wall structure of Lt is lost.
(B) Higher magnification within Lt. Oral bacteria (Ob) consist of organized cocci, bacilli and filamentous microorganisms.
(C) Within large tubule (Lt) closer to pulp, oral bacteria scarcely visible.

observed among the collagen fibrils (Fig. 2C).

Where most of the incisal dentin had been exposed to the oral cavity by occlusal wear, the large tubules opened out onto the dentin surface (Fig. 3A). The central area of the exposed dentin showed a greater angle of concavity than the peripheral dentin. The central area of the exposed dentin surface was covered with dental plaque, which was predominantly composed of cocci (Fig. 3B). In these cases, oral bacteria were observed in the vicinity of the open ends of the large tubules, although no bacterial invasion was seen inside the peripheral dentinal tubules. Bundles of collagen fibers and spherical bodies were scarce in those regions where such bacteria were observed. Furthermore, the wall-like structure of the large tubules faded out in these regions (Fig. 4A). The bacteria invading the interior of the large tubules consisted of cocci, bacilli and filamentous microorganisms (Fig. 4B). Oral bacterial invasion of the large tubules reached approximately 250μm from the open ends. It was not observed, how-

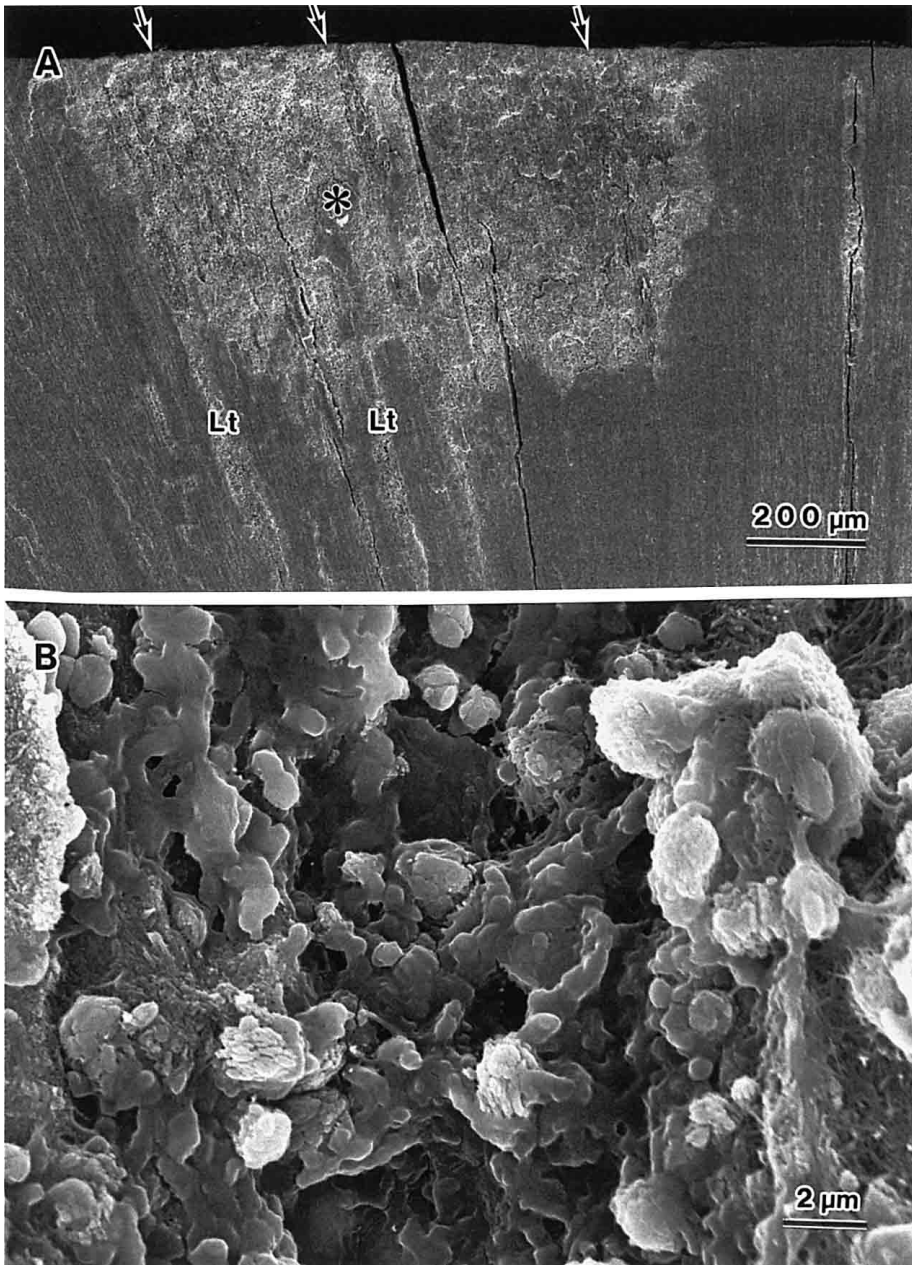

Fig. 5 FESEM photographs showing more advanced stage of attrition
(A) Low magnification of micrograph. Brighter region (asterisk) observed at occlusal dentin (arrows), and large tubules (Lt), which usually exist in this region, are indistinct.
(B) Higher magnification of brighter region. Numerous bacteria and dentinal debris.

ever, in the pulpal side, where collagen fiber was coarse (Fig. 4C).

Furthermore, where attrition was advanced, a cone-shaped caries pattern was observed near the incisal edge, with the base at the incisal edge and the blunt point toward the pulp. The structure of the large tubules and the surrounding dentin matrix were indistinct on

the incisal side, while the original structure of the large tubules was partially maintained near the pulp cavity (Fig. 5A). In the area where the large tubules were indistinct, there was an intermingling of numerous bacteria and destroyed dentin matrices (Fig. 5B).

Discussion

Previous studies have shown the distribution, structure, and formation of large tubules[1–3,7]. However, little attention has been given to morphological changes occurring in large tubules.

We observed structural changes in large tubules by using FESEM to reveal whether large tubules acted as a direct conduit for bacterial infection when incisal dentin was exposed to the oral cavity.

This study yielded 3 findings concerning the structure of large tubules: 1) Some large tubules were clearly observed to have glossy walls. The inside of these large tubules was largely composed of bundles of collagen fibers and small spherical bodies of aggregated crystals. In such cases, attrition was minimal; 2) Some large tubules retained their external configuration, but showed a faded-out wall structure. Oral bacteria were present in this type, and bundles of collagen fibers and small spherical bodies were scarce. In these cases, attrition had caused the large tubules to open out onto the incisal dentin surface; 3) In some cases, it was difficult to distinguish between the large tubules and the surrounding dentin at the incisal edge. This type showed numerous bacteria and destruction of the dentin matrices. Here, attrition was found to be advanced.

These structural changes in the large tubules bore a similarity to the progression of caries. On the basis of these findings, we surmised that these caries-like changes in the large tubules, which run from the vicinity of the dentino-enamel junction at the incisal edge toward the pulp cavity, were closely related to attrition levels in dentin.

There are various types of dentin caries: carious lesions advancing from enamel into dentin, root caries lesions, caries under restorations, etc.[5]. Generally, dentin caries occurs subsequent to enamel caries or cementum caries. However, in this study we observed the direct caries-like destruction of coronal dentin matrices. Exposure of incisal dentin easily occurs by attrition in the human deciduous tooth[8]. When attrition reaches dentin, the central part of the exposed dentin becomes more concave than the peripheral dentin, where it is continuous with the enamel. This concavity of dentin induces accumulation of plaque. Tronstad[22] has also observed that dental plaque covering exposed dentin was uneven. The large tubules that open out at the center of incisal dentin due to attrition are often covered by plaque. Consequently, large diameter tubules may act as a conduit for bacterial invasion.

It was reported that the depth of bacterial penetration into dentinal tubules ranged from 10 to 150μm[18]. In this study, however, many cocci, bacilli and filamentous microorganisms were observed at a depth of 250μm from the incisal edge within large tubules in the coronal dentin. This difference in depth of bacterial penetration may have been caused by the diameter of the tubules. That is to say, the diameter of the large tubules was almost 10-times that of the dentinal tubules. Furthermore, there are many reports showing that degree of bacterial invasion and localization depends on type of bacterial species in dentin[4,9,16,17].

We have demonstrated that collagen fibrils within large tubules are usually made up of mostly type-1 collagen in human deciduous dentin[3]. One report found that some strains of *Streptococcus mutans* adhered to type-1 collagen, and that bacterial adherence was a prerequisite for infection in a susceptible host[19]. Therefore, we surmised that dentin caries might be caused by the invasion of large tubules by oral bacteria. These tubules area filled with bundles of collagen fibers, and we hypothesized that the bacteria might bind to this collagen.

It has been reported that dentin proximal

to the incisal edge has characteristics slightly different from dentin in other areas. And morphologic irregularities were observed in the central area of the incisal dentin[1]. Tronstad[21] reported a hypo-mineralized band, extending from the incisal tip of the dentin to the pulp horn, in intact teeth. We also observed a continuous vertical alignment of interglobular dentin under the incisal edge[2]. These findings are suggestive of dentinogenesis imperfecta in dentin beneath the incisal edge.

Bacterial invasion occurs along the large tubules which are exposed in the oral cavity. Furthermore, the destruction of dentin starts from the peritubular dentin structure adjacent to the large tubule, expanding to the intertubular matrix. Because incisal dentin shows imperfect calcification, particularly in interglobular dentin, destruction of the matrix occurs quickly. Consequently, in some cases, while the enamel remains intact, the underlying dentin is decayed. However, because reparative dentin forms rapidly in deciduous teeth, dental pulp is rarely affected.

Tronstad and Langeland[20] found bacteria in exposed dentinal tubules, in cracks in dentin. Furthermore, they showed that a caries-like process might develop in the exposed dentinal tubules. However, no necrotic pulp tissue was seen in deciduous teeth. Moreover, Lundy and Stanley[14] found that secondary dentin stopped the proliferation of bacteria.

Miller[15] reported that fractures in deciduous teeth were caused by large tubules, but we believe that it is rather bacterial invasion into the large tubules inducing dentin decalcification that leads to fracture in deciduous teeth.

In the recent years, various methods to protect dentin by bonding several restorative agents to the surface of exposed dentin have been reported[12,13,24]. We believe it would be more effective if these methods were applied before bacteria had penetrated the large tubules on dentin being exposed in the oral cavity.

This study has shown that large tubules may act as a conduit for dentin caries formation when incisal dentin is exposed by attrition. Therefore, we believe that it is necessary to treat exposed surfaces of dentin at an early stage in the process of attrition in order to prevent dental caries.

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