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<tr>
<td>Journal</td>
<td>Bulletin of Tokyo Dental College, 46(1-2): 7-15</td>
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<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10130/235">http://hdl.handle.net/10130/235</a></td>
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Original Article

Relationship between Large Tubules and Dentin Caries in Human Deciduous Tooth

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Received 20 April, 2005/Accepted for Publication 15 June, 2005

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. 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\(^1\). 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\(^\text{1,3}\).

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.

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,
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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 $20\mu m$, and was largely composed of bundles of fibers run-

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.
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. 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).

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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 250 μm from the open ends. It was not observed, how-
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 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.
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
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 aggre-
gated 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 destruc-
tion 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 resto-
ratings, etc. Generally, dentin caries occurs
subsequent to enamel caries or cementum
caries. However, in this study we observed the
direct caries-like destruction of coronal den-
tin matrices. Exposure of incisal dentin easily
occurs by attrition in the human deciduous
tooth. 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 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. In this study, however,
many cocci, bacilli and filamentous microor-
ganisms 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 localiza-
tion depends on type of bacterial species in
dentin.

We have demonstrated that collagen fibrils
within large tubules are usually made up of
mostly type-1 collagen in human deciduous
dentin. One report found that some strains
of Streptococcus mutans adhered to type-1 col-
lagen, and that bacterial adherence was a
prerequisite for infection in a susceptible host.
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\(^{2}\) 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 dentino-genesis 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\(^{2}\) 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\(^{4}\) 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\(^{2,13,24}\). 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.

References


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