Scanning Electron Microscopic Study on the Fibrillar Structures within Dentinal Tubules of Human Dentin

Maricela Garcés-Ortíz, DDS, MSc, PhD, Constantino Ledesma-Montes, DDS, MSc, PhD,* and José Reyes-Gasga, MSc, PhD†*

Abstract

Introduction: Pulp biology is central to the whole tooth, and knowledge on its microstructure is changing with new studies. This study presents certain microfibrillar structures found within the dentin tubules of human teeth connecting dentin tubules and odontoblastic processes. **Methods:** We analyzed the crowns of 30 noncarious, human teeth. They were fixed; demineralized; and, later, processed and reviewed by means of scanning electron microscopy. **Results:** In the predentin layer, we found numerous fine fibrillar structures connecting the odontoblastic process and the wall of the dentinal tubule. In the inner dentinal third, we observed structures forming a dense microfibrillar network of variable thickness and diameters. These microstructures were very thin and numerous in this area, and their number decreased as more external dentin levels were examined. **Conclusions:** According to the review of the literature and our findings, these microfibrillar structures may be an unrecognized support system that holds and secures the odontoblastic process within the dentinal tubule *(J Endod 2015;41:1510–1514)*

Key Words

Dentin, dentinal tubules, microfibrils, micromembranes, odontoblastic process

From the *Clinical Oral Pathology Laboratory, Facultad de Odontología and †New Materials Laboratory, Instituto de Física, Universidad Nacional Autónoma de México, Mexico City, Mexico.

Address requests for reprints to Dr Constantino Ledesma-Montes, Clinical Oral Pathology Laboratory, División de Estudios de Posgrado e Investigación, Facultad de Odontología, Universidad Nacional Autónoma de México, México, 04510 DF Mexico. E-mail address: cledezma@unam.mx

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Dentin is a mineralized connective tissue with a self-recovering capacity. Dentin is a complex structure composed by odontoblasts, their odontoblastic processes (OPs), dentin tubules (DTs), noncollagenous proteins, and mineralized collagen forming the main dentinal corps. Dentin is classified into the following 3 types: primary, secondary, and tertiary. Only the primary and secondary dentin forms the dentin tissue of the normal noncarious teeth. DTs enclose and protect OPs from environmental harmful stimuli, and OPs secrete the proteic matrix formed by collagen and noncollagen proteins mainly developing in a mineralizing protein complex (1).

Odontoblasts are dentin-forming cells; they are tall columnar cells located in the frontier between pulp tissue and primary dentin. Their functions include secretion of the dentin matrix proteins; they are responsible for the mineral deposition process (2); and they are involved in the transmission of stimuli from the external environment to the pulp, helping in sensitivity to painful stimuli (3, 4).

Since the first description by Tomes (5), the odontoblastic process is not a simple cytoplasmic extension of the odontoblast; it is located within the DT and secretes all the dentin proteic and nonproteic components involved in the dentinal biomineralization process. There are several structures composing the dentin, and the OP is an extremely complex biologic odontoblastic structure because of the cellular polarization; it is located within the DT and reaches different distances at different dentin levels. The peritubular dentin surrounds this important structure, and, finally, the intertubular dentin encloses both structures (6–16).

Some researchers stated they found OPs located in the predentin zone only (7). Others reported the presence of OPs within the first third of the dentin thickness (6, 8–11), and in other studies, OPs were observed in the outer third reaching the dentinoenamel junction (10, 12–15). Fox et al (16) studied OPs in the root of extracted human third molars and found they were in contact with the dentinocemental junction. Interestingly, in teeth from *Macaca mulatta* monkeys, Kelley et al (13) observed that OPs were present in both the inner and outer dentin thirds and that the dentinal middle third was devoid of these structures.

In an extensive retrospective review of the literature, we found that since 1962 Johansen and Park (17) described the presence of thin, sheetlike membranes at all the dentinal levels, except predentin. Afterward, several scientists studied dentin at the scanning electron microscopic level and observed similar structures (6–8, 16, 18–20). Microstructures described as bifurcation lateral branches and ramifications of the OP were observed to be associated with holes and fenestrations in the wall of the DT. In other studies, these structures were described close to the dentinoenamel junction and described as bifurcations of the DTs. Interestingly, results from other studies reported on a more or less complex microfibrillar network attached to the OPs within the DTs (6–8, 10, 13–16, 19–21).

The aim of this work was to study and describe a microfibrillar and sheetlike network connecting DTs and OPs observed at different levels within the DTs of human teeth.

Materials and Methods

We analyzed 30 caries-free human bicuspids from patients undergoing orthodontic treatment at the Orthodontics Clinic of the Postgraduate Studies Division in the Facultad de Odontología of the Universidad Nacional Autónoma de México, Mexico City,

Mexico. All patients 18 years old or older and parents of the patients 17 years old or younger signed a letter of consent, donating their teeth to our institution for research purposes only. The ethics committee of our institution previously approved the protocol. Upper or lower bicuspids were extracted with regional anesthesia and minimal trauma using Xylocaine with 2% epinephrine (Zeyco, Guadalajara, Jalisco, Mexico). Immediately, we separated the crowns of the extracted teeth from the roots, making a groove at the cementoenamel junction with a water-cooled, tungsten carbide bur and a high-speed handpiece. The final separation was made using a chisel and a hammer. Crowns were grooved in the mesiodistal direction, split in 2 halves, and immediately immersed overnight in Karnovsky fixative solution at 4°C. Then, they were rinsed in cacodylate buffer (pH = 7.4) and demineralized in 5% nitric acid aqueous solution. After critical point drying, crowns were mounted on aluminum stubs with colloidal silver, coated with a 20-nm-thick gold layer, and examined with a JEOL 2000 SEM (JEOL, Tokyo, Japan).

Results

Patients were 15–21 years old with a mean age of 17.7 years (±1.82 years standard deviation [SD]). The features of the dentinal tissue close to the pulp showed no differences with those previously reported. In the inner nonmineralized dentin, DTs contained OPs associated with numerous, thin, microfibrillar structures forming a dense network (Fig. 1*A*). In the inner dentinal third, 1 end of the microfibrils was attached to the OP, and the other extremity was connected to the DT wall. Spaces among these microfibers were small, and they were so numerous that they occupied a large portion of the DT, forming a dense net (Fig. 1*B*). In other areas, these fibers seemed to coalesce, becoming wider and forming thick structures and frequently looking like homogeneous sheetlike material (Fig. 1*C*). In the previously mentioned areas, OPs were always in close contact with this microfibrillar network and were restricted within the periodontoblastic space. The most common direction of these microfibrils was from the surface of the OP to the dentinal wall, and in other instances, these microfibrils attached to both opposite internal surfaces of the DT. We were not able to see limits among the fibrillar or sheetlike structures and peritubular dentin. We observed that the base of the microfibril attached to the dentinal wall seemed to form a continuous structure among the dentinal tissue and the surface of the OP.

In the different analyzed dentinal areas, the length, number, and diameter of the microfibrillar material varied widely. In the predentin area, microfibrils measured between 0.01- and 2.5-μm long with a mean of 1.09 μm (±0.63 μm SD). The microfibril diameter was between 0.03 and 0.5 μm with a mean of 0.33 μm (±0.33 μm SD); their number varied among 30 to 52 microfibers/10 μm^2 with a mean of 42 microfibers/10 μm^2 (±6.8 microfibers/10 μm^2 SD).

Microfibers located in the inner third of the dentin-analyzed areas measured among 0.04- to 3.6-μm long (mean = 2.35 μm, ±0.99 μm SD). Their diameter varied from 0.03–0.46 μm (mean = 0.32 μm, ±0.13μm SD), and their number varied among 34–40 microfibers per 10 μm^2 (mean = 36.6 microfibers per 10 μm^2, ±2.31 microfibers per 10 μm^2 SD).

In contrast, longitude of the microfibers located in the middle third was between 0.01 and 0.2 μm (mean = 0.07 μm, ±0.05 μm SD); the microfiber diameter was among 0.03 and 0.52 μm with a mean of 0.05 μm (±0.13 μm SD), and their number varied among 8 to 18 microfibers per 10 μm^2 (mean = 11.9 microfibers/10 μm^2; ±2.9 microfibers/10 μm^2 SD).

The scarce microfibers located in the external dentinal third were almost all broken, and we propose this measurement will give false data; the diameter was between 0.01 and 0.02 with a mean diameter of 0.011 μm (±0.007 μm SD). In addition, their number varied between 0 and

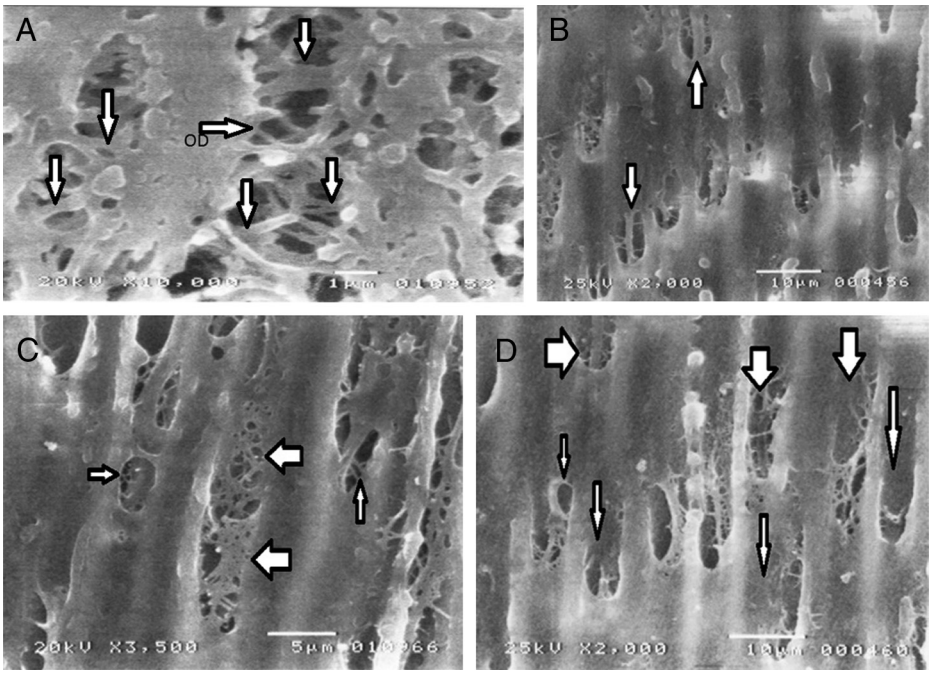

Figure 1. Nonmineralized dentin. (*A*) Multiple thin microfibrils of different diameters filling the dentinal tubules (*arrows*) (SEM, 10,000×. Scale bar = 1 μm). (*B*) The inner third of the dentin. DT showing the presence of microfibrillar structures connecting the odontoblastic process to dentinal walls (*arrows*). (SEM, 2000×. Scale bar = 10 μm). (*C*) DTs showing the presence of netlike structures formed by sheetlike structures (*wide arrows*) and some microfibrils (*thin arrows*). A hole is also seen (*small arrow*). (SEM, 3500×. Scale bar = 5 μm). (*D*) The middle third of the dentin. We can see a lesser quantity of microfibrils in this zone (*wide arrows*), and some areas of the dentinal tubules are empty (*thin arrows*) (SEM, 2000×. Scale bar = 10 μm).