Original Article

Diameter and Density of Dentinal Tubule in Human Primary Teeth

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Abstract

The aim of this study was to investigate dentinal tubule diameter and dentinal tubule density in primary teeth and to compare those values between each tooth type at electron microscope level. Thirty freshly extracted primary teeth were included in this experiment and categorized into six groups according to their tooth types and dental arches. All samples were first sectioned longitudinally, and then sectioned perpendicular to the direction of dentinal tubules which directed from the pulpal horn at three levels represented outer, middle and inner dentin. The result revealed that the diameter and density of dentinal tubule increased from outer dentin toward the pulp. The mean±SD of tubule diameter was largest in maxillary molar (1.44±0.24µm) and smallest in mandibular incisor (1.15±0.18µm). The density of dentinal tubules was greatest in mandibular incisor (58,080.33 tubules/mm^2) and lowest in maxillary molar (27,476.26 tubules/mm^2). The diameter of dentinal tubules tended to increase from anterior to posterior teeth, while, conversely, tubule density decreased from anterior to posterior teeth.

Keywords: Dentinal tubule, Primary teeth, Tubule density, Tubule diameter

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Introduction

The tubular structure of dentin makes it unique from other hard tissue in the body. The tubules are not only used for transportation of mineral salt to deposit at the calcified front at the mineral wall but also play an important role in transferring stimuli and irritants to nerve terminal at the surface of the dental pulp. After loss its coverage, dentin became permeable to environment, opened for invasion of bacteria and its toxin while it was having continuous outward flow to counter balance.[1]

The model composite restoration utilizes the porous property of dentin by desiring an adhesive to form a mechanical lock with decalcified dentine on etched surface and inside the tubule forming hybrid layer and resin tags. The density and diameter of dentinal tubules have strong relation with bond strength of dental adhesive.[2-4]

There is a possibility that primary teeth have different properties from permanent teeth. However, most of bond strength studies were experimented on permanent human teeth or even animal teeth. While primary teeth have many factors, that should be taking into account before making any restoration. Only few reports published on the diameter and density of dentinal tubules. Therefore, this study aimed to investigate the properties of primary dentin by measuring tubule diameter and the number of tubules per area at different depth of coronal dentin and comparing those values between each tooth type at electron microscope level.

Materials and Methods

This study has been approved by The Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University. All teeth specimens were collected under consent of participants and their parents.

Thirty freshly extracted prolonged primary teeth from 4-14-year-old children were included in this study. The tooth specimens must be intact or have one small proximal carious lesion extend no further than one-third of the dentin thickness.

The specimens were rinsed in running tap water immediately after extraction and stored at 4°C in normal saline solution with 0.1 % thymol until used.

All samples were divided into 6 groups according to their tooth types and dental arches; maxillary incisors (n=6), maxillary canines (n=4), maxillary molars (n=5), mandibular incisors (n=7), mandibular canines (n=3), and mandibular molars (n=5).

The roots were cut off at the level of 1 mm apical to cementoenamel junction (CEJ) by using a cylinder diamond bur (Intensive®, Swiss Dental Product, Switzerland) attached on an air rotor handpiece with water spray cooling. The remaining pulp tissues were removed by using endodontic barb broach.

Tooth preparation procedure for incisors and canines were similar. Crowns were cut longitudinally in buccolingual direction through the middle part of the crowns in order to gain access to the most straight dentinal tubules running from the pulpal horn to the dentinoenamel junction (DEJ) as shown in Fig. 1A.

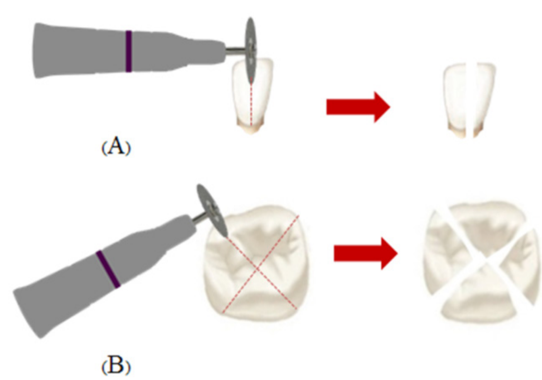

Figure 1 *Tooth specimen preparation in anterior tooth (A) and posterior tooth (B)*

In order to measure the most straight dentinal tubules which run from the tip of each pulpal horn to the tip of the cusp in posterior teeth, crown of posterior tooth having three to four pulpal horns were cut longitudinally in two directions from mesiobuccal line angle to distolingual line angle and from distobuccal line angle to mesiolingual line angle (Fig. 1B).

In addition, all specimens were sectioned perpendicular to dentinal tubules at 1/4, 1/2, 3/4 of its thickness to represent outer, middle and inner dentin, respectively (Fig. 2).

The cut tooth surface were cleaned by stored the whole specimen in 5.25 % sodium hypochlorite solution for 12 hours then placed into an ultrasonic cleaner for 10 minutes. The samples were left dried at room temperature in clean ventilation storage for 24 hours. The specimens were attached to the stub with conductive adhesive and coated with gold-palladium under vacuum in sputter coater equipment (JEOL® JFC1200 Fine Coater; JEOL, Tokyo, Japan).

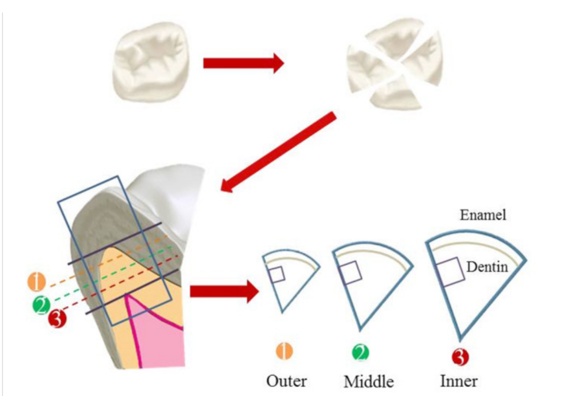

Figure 2 *Dentinal tubules over the pulpal horn at each dentin level were examined by SEM.*

The sample was examined under scanning electron microscope (SEM) (JEOL® JSM 6610LV, JEOL Ltd., Tokyo, Japan) at 15kV. The SEM images of the same dentinal tubules over the pulp horn at each level were captured at magnification of x3000 into the digital image files for further analysis.

The tubule diameter was measured by using the image analysis software, Image J (NIH, USA) from the digital images compare to the calibration bar. The formula proposed by Forssell-Ahlberg et al. (1975)[5] was used to calculate the number of dentinal tubules/mm^2.

$$X = \frac{10^6}{(l/i)^2} \cdot n$$

Where X = number of tubule /mm^2

l = length of side of photomicrograph (μm)

i = magnification

n = number of dentinal tubules on the photomicrograph

Every dentinal tubules presented in the SEM images was counted excluding those tubules that smaller than semi-circle at the edge of images and those tubules with branched at the outer dentin. To minimize the error results from sectioning, the shortest part of ovoid tubule was used to represent the diameter of that tubule.

Seventy-seven dentinal tubules which was the least counted number of the tubules on the SEM images at x3000 magnification from each group was selected to statistically compare all samples groups. The results were analyzed by using statistical analysis program (SPSS version 22.0, SPSS Science, Chicago, USA). The difference in means was analyzed by two-way ANOVA and Tukey's test pairwise comparison (p<0.05).

Results

Representative SEM images of dentinal tubules in primary teeth at different dentin depths were shown in Fig. 3.

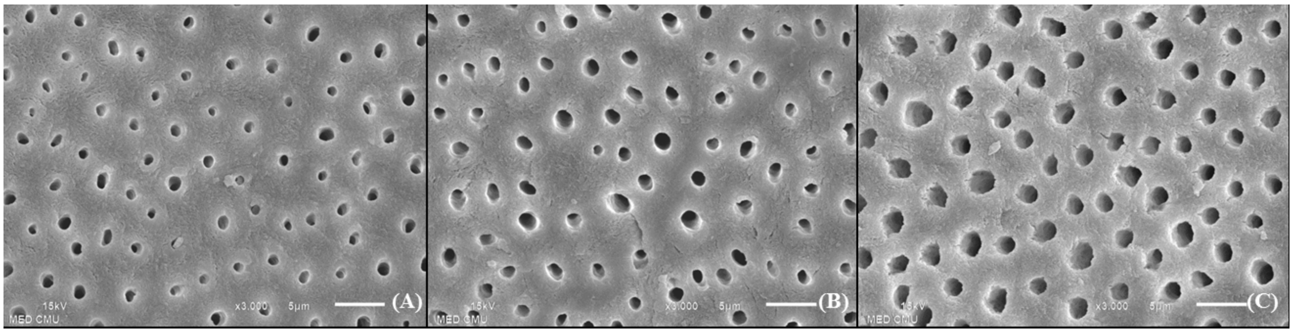

Figure 3 *Samples of SEM images of dentinal tubules in outer (A), middle (B), and inner (C) dentin of primary maxillary lateral incisor*

The mean±SD value of tubule diameter in each tooth type was shown in Table 1. The diameter of dentinal tubule increased significantly (*p*<0.05) from outer dentin toward the pulp in all tooth type. Overall, the diameter of dentinal tubules in inner dentin were larger than the middle dentin 6.72-22.02 % and larger than the outer dentin 23.33-55.96 %. Regarding tooth types, Molars had significant larger tubule compared to mandibular incisors and canines. The largest mean tubule diameter was maxillary molar and the smallest was in mandibular incisor.

Table 1 *Dentinal tubule diameter by tooth types at different depth*

Tooth type	Mean±SD of tubule diameter (μm)			
	Outer	Middle	Inner	Average
Maxillary incisor (n=6)	$1.09{\pm}0.04^{a}$	$1.33{\pm}0.04^{b}$	$1.70{\pm}0.04^{c}$	1.37±0.25
Maxillary canine (n=4)	$1.19{\pm}0.36^{a}$	$1.27{\pm}0.13^{b}$	$1.59{\pm}0.16^{c}$	1.35±0.30
Maxillary molar (n=5)	$1.24{\pm}0.17^{a}$	$1.37{\pm}0.11^{b}$	$1.69{\pm}0.14^{c}$	1.44±0.24
Mandibular incisor (n=7)	$0.96{\pm}0.04^{a}$	$1.09{\pm}0.02^{b}$	$1.39{\pm}0.05^{c}$	1.15±0.18
Mandibular canine (n=3)	$1.20{\pm}0.16^{a}$	$1.36{\pm}0.13^{b}$	$1.48{\pm}0.17^{c}$	1.34±0.19
Mandibular molar (n=5)	$1.19{\pm}0.15^{a}$	$1.42{\pm}0.14^{b}$	$1.67{\pm}0.10^{c}$	1.43±0.24

**The different superscript character (a,b,c) in the same row indicates significant different at p<0.05 when analyses by two-way ANOVA and Tukey's test pairwise comparison.*

The result of tubule density or the number of tubules per unit area was shown in Fig. 4. Mandibular incisor had the highest average dentinal tubules density while maxillary molar had the lowest number. The range of tubular density at outer dentin was 19,800.51-50,112.40 tubules/mm^2 and at the inner dentin was 35,787.59-64,412.77 tubules/mm^2. The tubular tended to increase from outer to inner layer of dentin in all tooth type.

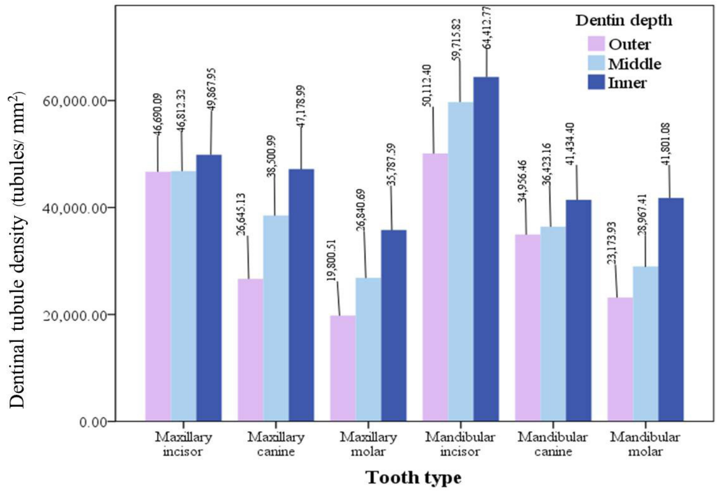

Figure 4 *Dentinal tubule density at different dentin depth in each tooth type (tubules/mm2)*

Discussion

In general, the diameter of dentinal tubule obtained from this study was relatively larger than other studies[3,6,10] even though this study used no acid or chelating agent which might enlarge the size by dissolving mineral content of peritubular dentin.

In more detail, our tubule diameter of primary incisors was larger than the result reported by Costa *et al.*, 2002.[6] At the middle of dentin, the mean tubule diameter of this study was 1.33 μm in maxillary incisor and 1.09 μm in mandibular incisor while of those study was 1.05 μm in general. However, the mean tubular diameter in canine was smaller than the result obtained by Sumikawa *et al.*, (1999).[7]

Schilke and colleague (2000)[8] and Lenzi and colleague (2013)[9] reported larger dentinal tubule in primary molars at middle and inner dentin compared to this present study. However, the opposite results Koutsi *et al.*, 1994 and Ruschel *et al.*, 2012 reported smaller dentinal tubule (0.96-1.29 μm, 0.794-1.00 μm, respectively) compare with this study.[3,10]

The result from this study supported the fact that the number of dentinal tubules per unit area was increasing with depth. The outer dentin had less number of dentinal tubules per unit area than the middle and inner dentin.

The tubule density of incisors and canines found in this study (Fig. 4) were greater than the founding of Costa *et al.*, 2002 (9,641-23,114 tubules/mm^2)[6] and Sumikawa *et al.*, 1999 (approximately 26,000-35,000 tubules/mm^2)[7] respectively. The tubule density of molars found in this study (Fig. 4) were greater than the results from other investigations by Koutsi *et al.*, 1994 (17,433-26,391 tubules/mm^2)[3], Schilke *et al.*, 2000 (18,243-24,162 tubules/mm^2)[8], and Ruschel *et al.*, 2012 (17,997.60-25,211.32 tubules/mm^2)[10] while lower than Lenzi and colleague, 2013 (85,541-171,510 tubules/mm^2) which included canaliculi in their study.[9]

The different area of investigation might affect both diameter and density of the dentinal tubules.[11] Focusing in measuring the straight dentinal tubules travelling from the pulpal horn to either the tip of incisor or the tip of the cusp could be the reason for the higher number of tubules/mm2 in our study compared to other studies.

The average and standard deviation of remaining dentin thickness from all tooth specimens in this study was 2.35±0.23 mm. The SD value indicated that the collected specimen had small difference in tooth age when it had been extracted.

The difference in diameter and density of dentinal tubules in each tooth types and the depth of dentin will determine the hydraulic conductance value of dentin which allows dentinal fluid to flow in and out at ease. The result from the present study and from previous studies suggested that the increase of diameter density of dentinal tubules related to higher hydraulic conductance of dentin.[4,14-15]

The dentinal tubule diameter and density over the pulpal horn increased significantly among 3 levels of dentin. The results can be explained by the convergence of tubules as they approach the pulp.[9,12-13]

The bond strength studies were mainly experimented on permanent human teeth or animal teeth and the result was implied to use for primary human teeth which may have different in morphology and properties.[16,17]

Our studies provided basic knowledge of dentin characteristic and properties in primary teeth. However, percentage of area which occupied by dentinal tubules and peritubular dentin would be investigated in future.

Conclusion

The diameter and the density of dentinal tubules increased from outer dentin toward the pulp in all tooth type of primary teeth.

The diameter of dentinal tubules in primary teeth tended to increase from anterior to posterior teeth, in opposite, tubule density decreased from anterior to posterior teeth.

Acknowledgement

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