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Dentin tubule numerical density variations below the CEJ

T. Komabayashi*, **G. Nonomura**, **L.G. Watanabe**, **G.W. Marshall Jr.**, and **S.J. Marshall**
Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, 707 Parnassus Avenue, D2246, University of California, San Francisco, CA 94143-0758

Abstract

Aim—To evaluate dentin tubule numerical density variations below the CEJ.

Methodology—Three human non-carious permanent canines were sectioned parallel to the CEJ to obtain dentin disks 1 mm thick whose surfaces were 1 mm and 2 mm below the CEJ. Each disk was sectioned into quarters resulting in four segment locations: facial, lingual, mesial, and distal. The outer (PDL side) and inner (pulp side) surfaces of the specimens were shaped to expose dentin with SiC papers and polished. Numerical tubule density was determined from SEM images. All data were statistically analyzed using a three-way ANOVA.

Results—The dentin tubule density (number/mm^2) ranged from 13,700 to 32,300. Dentin tubule density was relatively uniform at 1 and 2 mm below the CEJ and increased by a factor of about two from the outer to the inner surface, which was significantly different ($P<0.0001$).

Conclusions—The tubule density variations at the cervical root did not present the marked changes.

Keywords

Cemento-Enamel Junction (CEJ); Cervical lesions; Density; Dentin hypersensitivity; Dentin tubules; Root dentin; Scanning Electron Microscopy (SEM)

Introduction

Dentin hypersensitivity is defined as a transient pain arising from exposed dentin, typically in response to chemical, thermal, tactile, or osmotic stimuli, which cannot be explained by any other dental defect or pathology [1]. Dentin exposure can occur due to trauma, gingival recession, and various restorative procedures [2]. Dentin hypersensitivity is predominately located on the cervical part of the buccal surface [3–5], although dentin hypersensitivity can occur on all tooth surfaces [5]. According to Brännström, an increased number of exposed dentin tubules can result in increased dentinal fluid movement and patient discomfort [6]. The high patency of the tubules on the outer dentin surface in dentin hypersensitivity teeth was demonstrated by scanning electron microscopy (SEM) [7–11].

Dentin is an anisotropic biological composite and exhibits regional differences in tubule density [12,13]. With respect to the location from the CEJ, Mjör and Nordahl demonstrated that the

*Takashi Komabayashi DDS, MDS, PhD, Assistant Professor, Department of Endodontics, Texas A&M Health Science Center Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246 USA, TEL: 214-828-8365, FAX: 214-874-4507, E-mail address: tkomabayashi@bcd.tamhsc.edu ICD38719@nifty.com.

mean number of dentin tubules in the middle part of the root was significantly lower than in the middle part of the crown [14]. Harran Ponce et al. observed that the numerical density of dentinal tubules is greater in the crown, decreasing toward the apical third of the root. In that study, numerical density of dentin tubules at the external dentin surface was reported to be 21,000, 15,000, and 13,000 per mm^2 at crown-root junctions, cervical-middle, and middle-apical thirds of the root, respectively [15]. Dentin tubule numerical density in root dentin has been studied by various investigators [14,16–20]. However, dentin tubule numerical density information which is focused on the cervical part of the tooth, located just below the CEJ, is scarce.

The dentin localized in the close proximity to the CEJ is an area which often develops dentin hypersensitivity and is subject to Class V root caries lesions, sclerotic non-carious lesions, and abfraction lesion [21–28]. It has been reported that non-carious cervical lesions occur in up to 85 per cent of individuals, with their prevalence and severity increasing with age [28]. Large non-carious cervical lesions with exposed dentine can increase the risk of pulp exposure or tooth fracture [21]. Fractures may occur in cervically-notched teeth, severing the crown of the tooth in anterior teeth, whereas in the cusp in posterior teeth [24]. Canine is common for class V root caries and cervical lesions because it retains longer than other teeth and locates at the transition from anterior to posterior.

The aim of this study was to evaluate dentin tubule numerical densities 1mm and 2 mm below the CEJ.

Materials and Methods

Specimen preparation

The specimens used in this study were prepared from permanent human non-carious, erupted, and fully root-formed maxillary or mandibular canines. All the teeth were recently extracted from patients needing extractions as a part of their dental treatment, as approved by the UCSF Committee on Human Research. Teeth were sterilized by gamma irradiation and refrigerated at 4°C in Hank's balanced salt solution (HBSS) prior to use [29]. Figure 1 shows a schematic of the specimen preparation and study procedures. Three teeth were sectioned parallel to the CEJ with a low speed water cooled diamond saw (Buehler, Ltd., Lake Bluff, IL) to obtain dentin disks 1 mm thick whose surfaces were 1 mm and 2 mm below the CEJ. One disc was obtained per tooth. Each disc was sectioned into quarters, resulting in four specimens (facial, lingual, mesial, and distal). Twelve specimens were obtained from three teeth. The outer (PDL side) and inner (pulp side) surfaces of the specimens were shaped to expose dentin with 800 and 1200 grit SiC papers (ALLIED, Rancho Dominguez, CA) and polished using 6, 3, 1, and 0.25 µm diamond suspension (Buehler, Ltd., Lake Bluff, IL). The specimens were ultrasonicated for 5 seconds in deionized water after each polishing step to remove any polishing debris and help insure that all tubules would be visible. To remove any remaining smear layer, 0.5 M EDTA was applied for 30 seconds, rinsed with deionized water, and gently dried by compressed air to remove excess water, but the surface was left moist.

SEM analysis

SEM images (2000x) were collected from 12 locations per specimen (i.e. outer/inner, left/center/right, 1 mm/2 mm) using the CFAS “wet” mode (ISI ABT SX-40A SEM, Topcon Instruments, Pleasanton, CA). In this mode, neither drying nor sputter coating is necessary. As summarized in Figure 1, the orientations and intratooth positions were chosen so the tubules would be roughly perpendicular to the surface examined, yielding circular cross-sections of the tubules. Images were stored digitally and analyzed with image analysis software (Ultrascan 2.1.1, Soft Imaging Software, Kevex Sigma, Noran Instruments, Inc., Madison, WI).

As indicated above, a tooth yielded dentin specimens at multiple sites, varying by depth (1 mm, 2 mm), location (left, center, right), and position (outer, inner) in four specimens (facial, lingual, mesial, and distal). Overall, 144 SEM images were obtained from 3 teeth. The number of tubules was counted manually on each SEM image and converted to tubule density, the number of tubules per square mm (mm^2). Tubules that were partially in an image were counted as a half of a tubule.

Statistical Analysis

The tubule densities by location (left, center, right) were averaged because there were no statistically significant differences in these values. Mean and standard deviation (S.D.) of the dentin tubule densities (tubules/ mm^2) were calculated. The coefficient of variation (CV) was calculated. All data were statistically analyzed using a three-way ANOVA comparing tubule density as a function of depth, location, and internal/external position. (Statview5, SAS Institute Inc, Cary, NC)

Results

Figure 2 shows composite 4 SEM images, representative of the 144 SEM images observed. Images were classified by depth (1 mm, 2 mm) and position (outer, inner) in four regions (facial, lingual, mesial, and distal). Tubule patterns appeared similar in the images from the facial and lingual sections and in those from the mesial and distal sections. However, the facial and lingual images differed from the mesial and distal images in terms of tubule roundness, which was due to tubule orientation.

The dentin tubule densities and their standard deviations (tubules/mm^2) are summarized in Table 1. The dentin tubule density (number/mm^2) ranged from 13,700 to 32,300. The CV ranged from 0.08 to 0.22. Dentin tubule density was relatively uniform at 1 and 2 mm below the CEJ. However, dentin tubule density was increased by a factor of about two from the outer to the inner surface, which was significantly different ($P<0.0001$). P values for other variables were 0.08 for region (facial, lingual, mesial, and distal) and 0.10 for depth (1 mm, 2 mm). Accordingly, no statistical significance was found among four regions (facial, lingual, mesial, and distal). No statistical significance was found between two depths (1 mm, 2 mm).

Discussion

In preparing our samples, we employed the polishing technique previously used by Oliveira et al. [30], intended to render the dentin surface as devoid as possible of a smear layer. Permanent canines were used because their size was thought to be suitable for specimen preparation when divided into four pieces as described in Figure 1 and they allowed examination of outer and inner areas in the same tooth. In addition, canine teeth commonly develop class V root caries and cervical lesions because they are retained longer than other teeth and are located at the transition from anterior to posterior segments. However, the extremely small specimen size in this study made polishing very difficult, which resulted in some remnant smear layer. To remedy this, EDTA was applied to remove the smear layer entirely before SEM observation. EDTA was chosen because it has been reported to cause less demineralization of the dentin structure than other methods [31]. Because the EDTA caused an increase in the tubule diameter, it was not possible to measure tubule diameter and compare tubule openness using such categories as plugged, partially open, and open [32]. However, the tubules near the pulp chamber did appear to have larger diameters than those near the outer surface, as expected. To ensure an accurate count, considerable care was taken to produce specimens from a variety of locations with tubule orientation and surface conditions. Distributions with CV < 1 were considered low-variance. In this regard, the number of specimen in this study was appropriate.

The tooth is longer facial-lingually than mesial-distally, so the odontoblasts in those regions had to travel further to reach the pulp chamber. Therefore, we expected to find greater tubule density on facial-lingual sides. This was indeed the trend; however, it was not statistically significant.

With respect to the location from the CEJ, Mjör and Nordahl demonstrated that the mean number of dentin tubules in the middle part of the root was significantly lower than in the middle part of the crown [14]. Harran Ponce et al. observed that the numerical density of dentinal tubules is greater in the crown, decreasing toward the apical third of the root. In that study, numerical density of dentin tubules at the external dentin surface was reported to be 21,000, 15,000, and 13,000 per mm^2 at crown-root junctions, cervical-middle, and middle-apical thirds of the root, respectively [15]. Therefore, we expected to find gradual changes in the numerical density of the dentinal tubules. However, our study found no statistically significant difference between 1 mm and 2 mm below the CEJ. This may be due to the narrow area targeted by the study, even though this is the area where cervical root exposure is frequently observed in relation to dentin hypersensitivity. Future research should examine distances of 3 mm and greater from the CEJ toward the root apex so as to have a wider targeted area at cervical root dentin. This study also suggests for further study of tubule density of teeth other than the canines, though the specimen preparation will be more difficult and complex. Such research will provide a useful database to increase knowledge of the nature and causes of dentin hypersensitivity.

Dentin tubule density increased from the outer to the inner surface by a factor of two in our study. This result agrees with the report by Pashley et al. that tubule density increased as the pulp chamber was approached [33]. Similarly, Harran Ponce et al. observed that the numerical density of dentin tubules at the internal and external dentin surface at crown-root junctions was 38,000 and 21,000 per mm^2, respectively [15]. In regards to comparison between root and coronal dentin, Fogel et al. found that the ratio of the number of tubules per unit area at the pulp and outer root surface was only about 2:1 rather than the 4:1 found in coronal dentin [18]. Mjör and Nordahl also reported that differences in the density of tubules between the peripheral and inner aspects were more marked in the crown than in the root [14]. In the current study, the tubule density variations at the cervical root did not present the marked changes observed in coronal dentin studies. The transition from coronal to root dentin around the pulp chamber needs further study.

The dentin localized in the close proximity to the CEJ is an area which often develops dentin hypersensitivity and is subject to Class V root caries lesions, sclerotic non-carious lesions, and abfraction lesions [21–28]. Although the mechanisms of pain transmission across dentin are not fully understood, dentin hypersensitivity is reduced when the dentin tubules are occluded [34,35]. The findings of this study provide better understanding of the anatomy of dentin as it pertains to dentin hypersensitivity. The results of this study provide useful information for dentin as a substrate that needs treatment for dentin hypersensitivity, carious, and non-carious lesions. Since dentin is a substrate whereas material is an adherent, the results of this study provide useful data for future dental material research and development to occlude exposed dentin tubules in dentin hypersensitivity treatment.

Conclusion

The following conclusions can be drawn from this study for canines.

1. The dentin tubule density (number/mm^2) ranged from 13,700 to 32,300.
2. Dentin tubule density was relatively uniform at 1 and 2 mm below the CEJ.

3. Dentin tubule density was about two times greater at the inner surface than at the outer surface.
4. There was no difference in tubule density with region (facial, lingual, mesial, and distal) or depth for the areas studied.
5. The tubule density variations at the cervical root did not present the marked changes.

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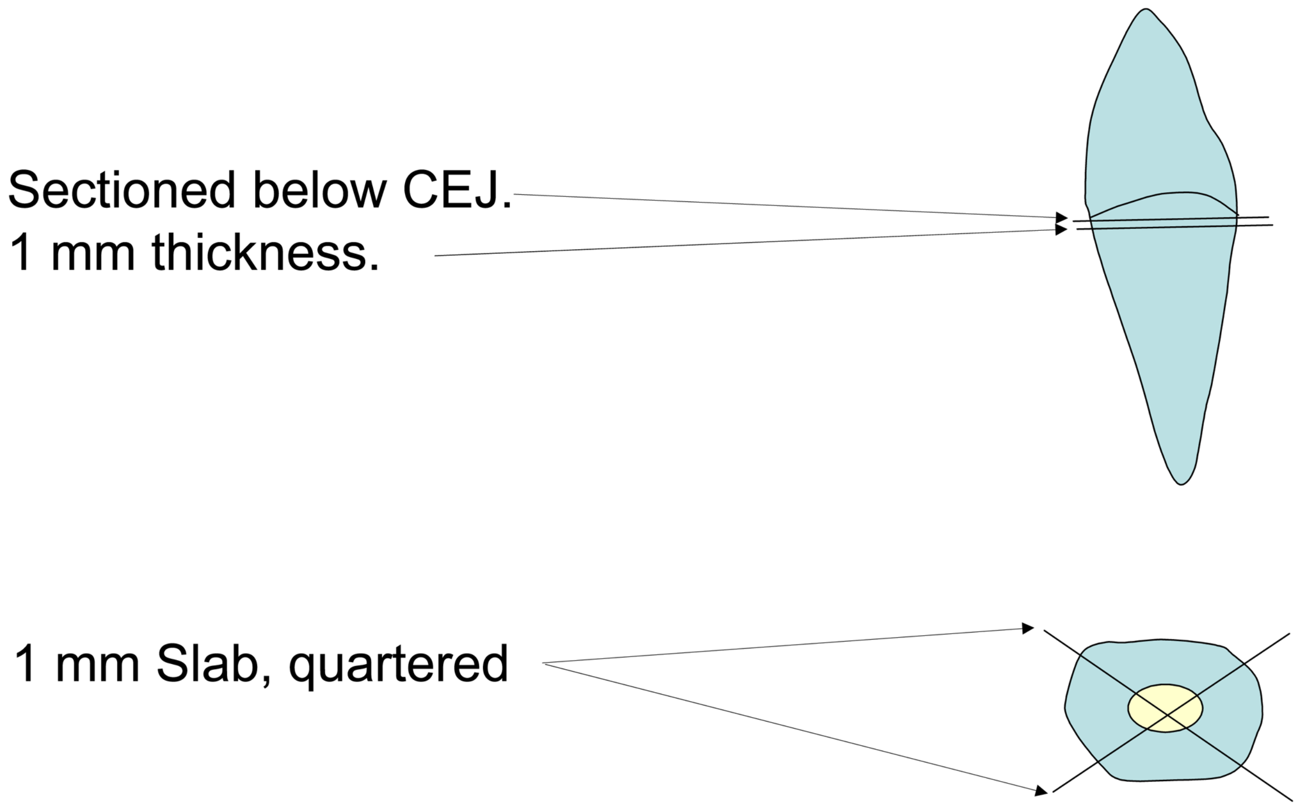
Sectioned below CEJ.
1 mm thickness.
1 mm Slab, quartered

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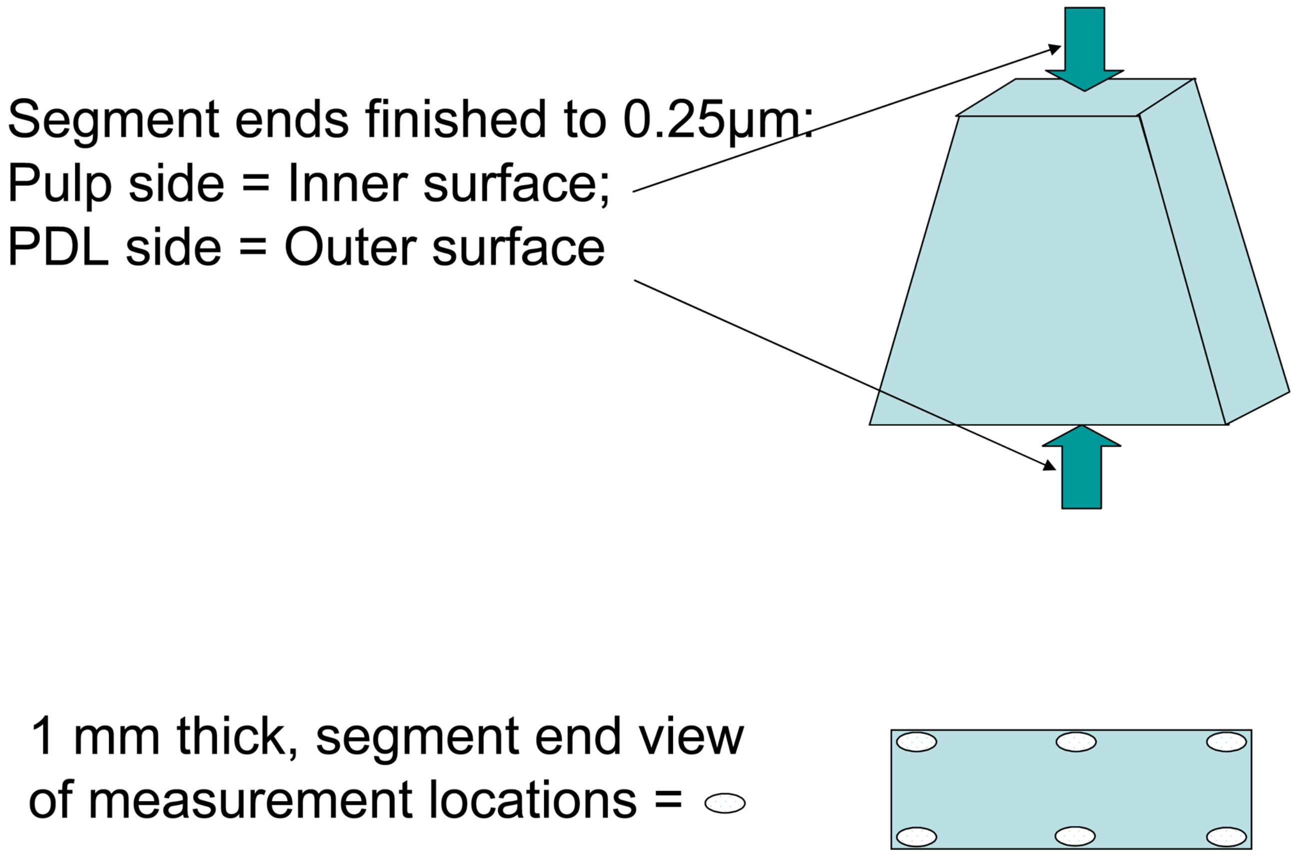

Figure 1.
Schematic of sample preparation and study procedures.

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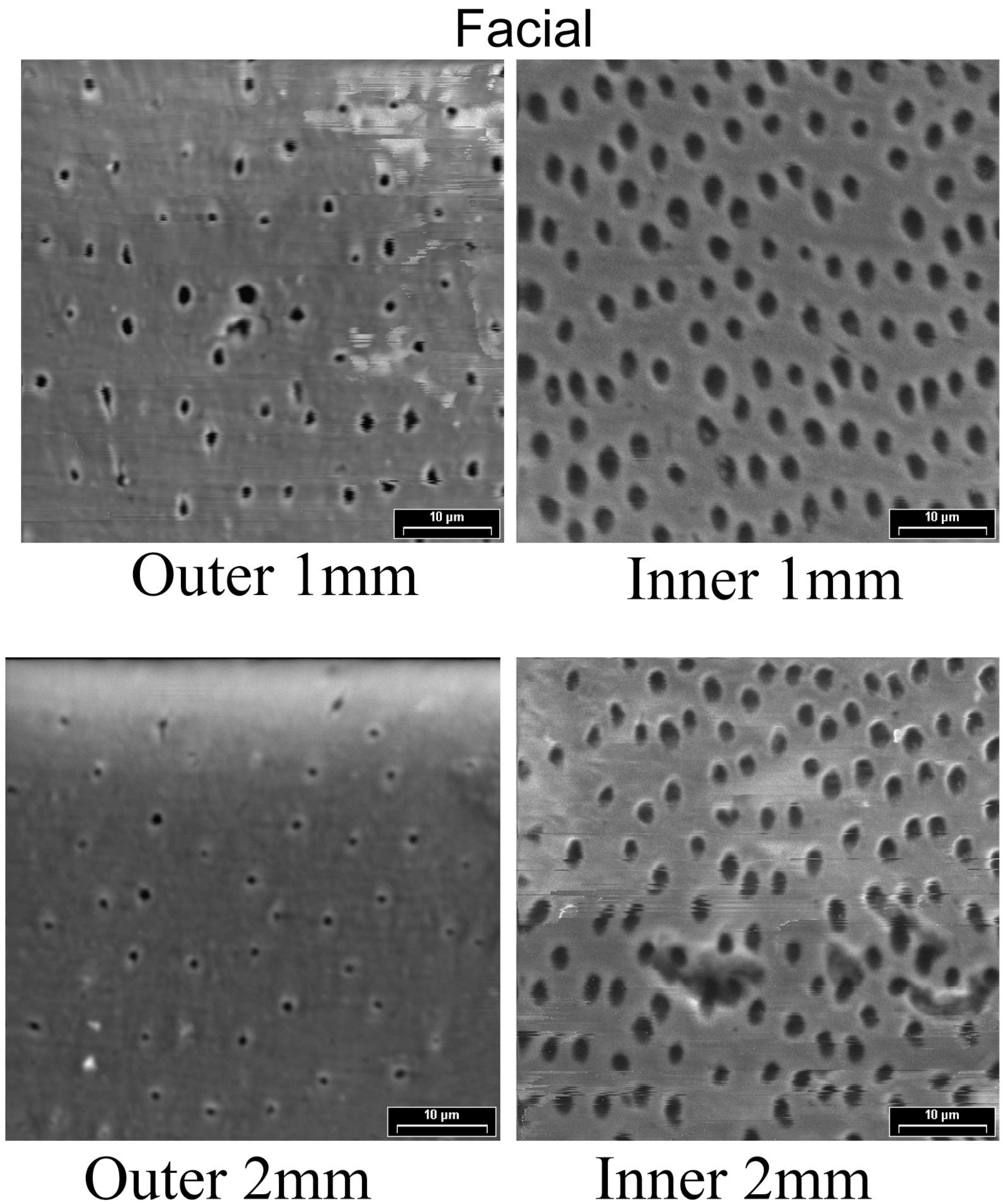

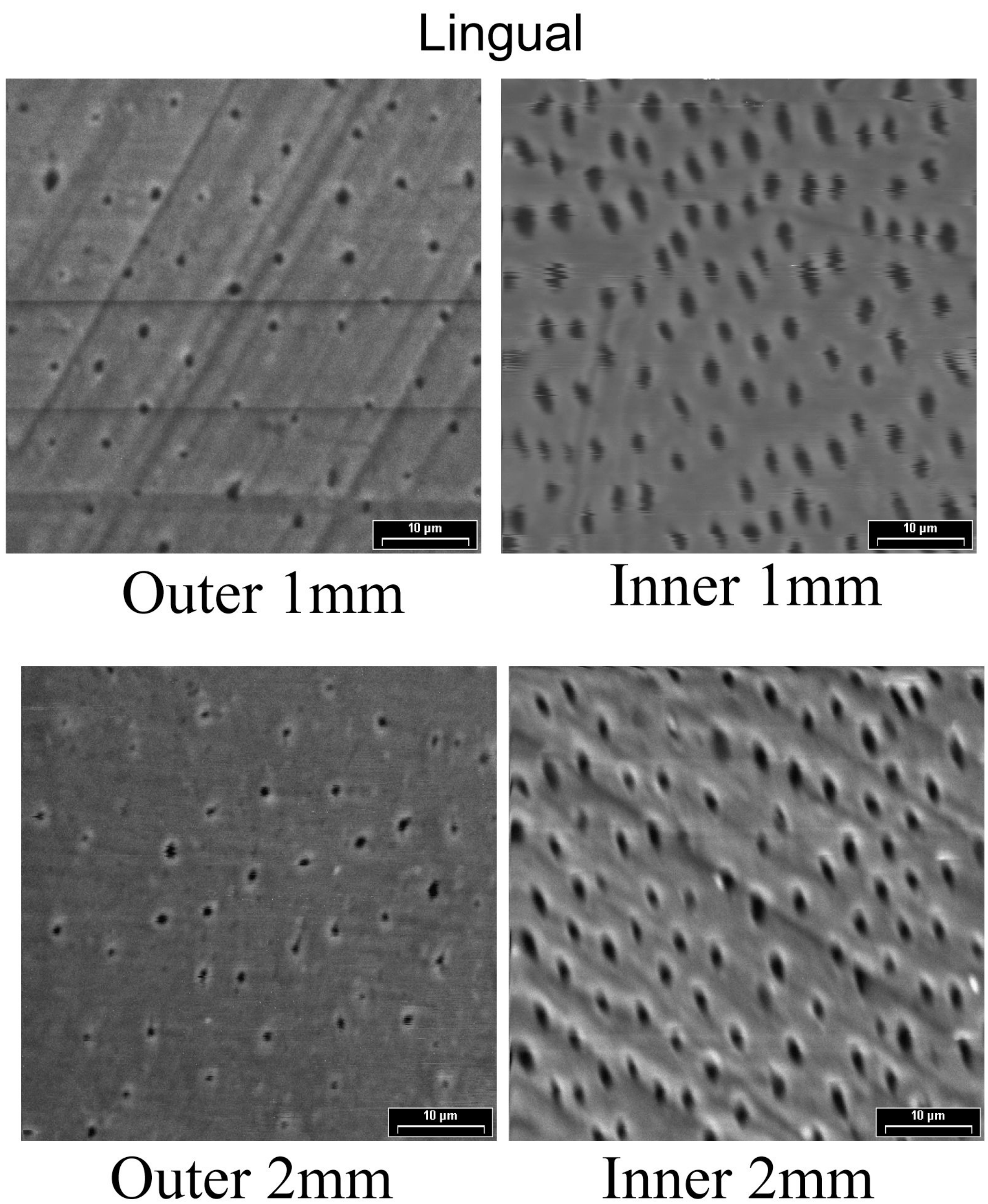
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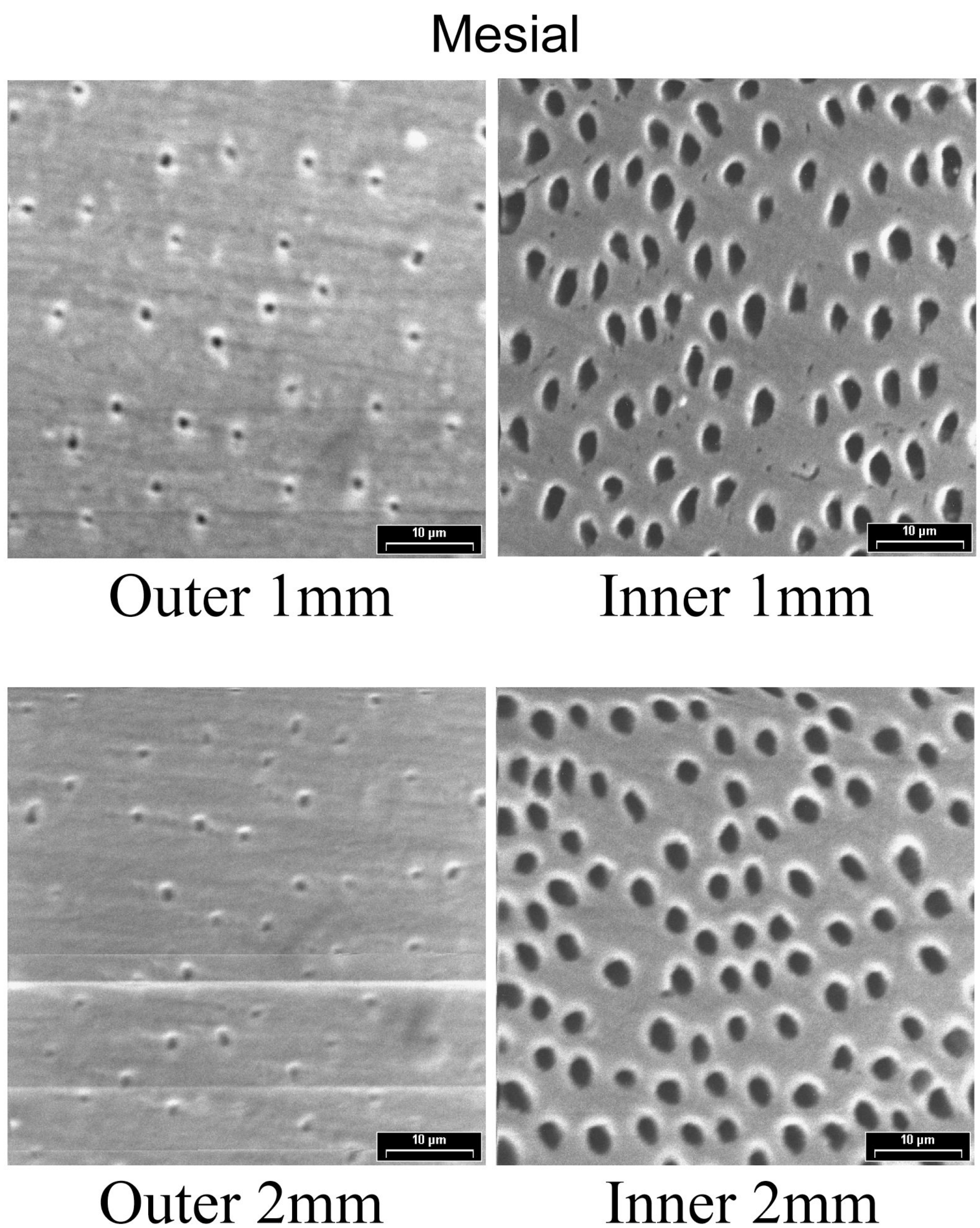
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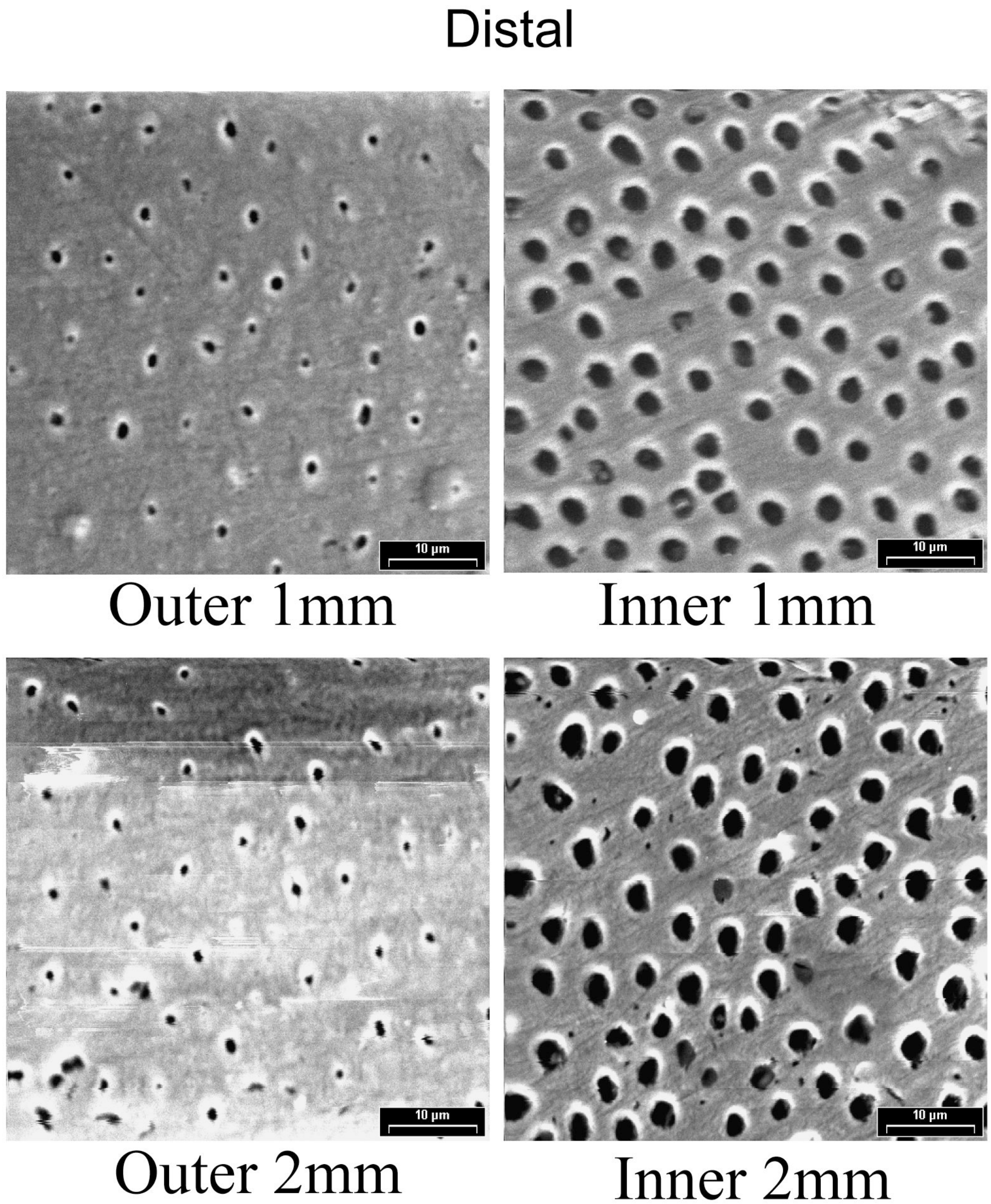

Figure 2.
SEM images

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Table 1

The dentin tubule densities (mean (standard deviation) tubules/ mm2, CV)

		Buccal	CV	Lingual	CV	Mesial	CV	Distal	CV
Outer	1 mm	16300(1300)[a]	0.08	16800(2200)[a]	0.13	13700(1800)[a]	0.13	15000(1700)[a]	0.11
	2 mm	15200(1400)[a]	0.09	14700(1500)[a]	0.10	16500(2600)[a]	0.16	16200(3500)[a]	0.22
Inner	1 mm	31400(5100)[b]	0.16	28400(2800)[b]	0.10	25300(2900)[b]	0.11	28700(4200)[b]	0.15
	2 mm	32300(2600)[b]	0.08	31600(4400)[b]	0.14	26000(3400)[b]	0.13	29100(4700)[b]	0.16

Groups identified by different superscript letters are significantly different ($P<0.0001$).