The Effect of Age on Bacterial Penetration of Radicular Dentin

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Abstract

The aim of this study was to determine the effects of patient’s age on the prevalence and depth of bacterial penetration inside dentinal tubules. Fifty-six single-rooted teeth were divided in two groups based on the patient’s age: young (ages 18–25) and old (age ≥60). Teeth were instrumented and inoculated with a standardized bacterial load and incubated for 20 days. Histological analysis was performed to determine the degree of infection of the dentinal tubules by counting the number of invaded tubules and the depth of penetration of bacteria inside the tubules. A significantly higher number of tubules were invaded by bacteria in the young group compared with the old group (p = 0.014). Also, the depth of invasion by bacteria was significantly higher in the young than in the old group (p = 0.033). These results suggest that bacterial infection of dentinal tubules occur to a lesser extent in older patients. (J Endod 2009;35:78–81)

Key Words

Age, bacterial penetration, dentin, dentinal tubules, endodontics

Bacteria are the cause of apical periodontitis that infect the dental pulp and dentinal tubules (11). Once they invade the tubules, they may be responsible for persistent root canal infection (2, 3). Past studies have examined the depth of bacterial penetration into radicular dentin (4–7) or penetration of bacteria into dentinal tubules in general (2). However, the influence of the age of the patient was not considered.

In one study, it was shown that Streptococcus sanguis migrated into dentinal tubules at a depth of 792 μm and that it penetrated approximately 50 μm more in immature teeth than in mature teeth (8). Another in vitro study (9) has shown that S. sanguis could penetrate into dentinal tubules a mean of 382 μm, whereas Prevotella intermedia could penetrate to a mean of only 26 μm. In an examination of 97 clinically nonvital teeth, Shovelton et al. (10) found that 63% of teeth showed bacterial penetration into the radicular dentinal tubules. However, the depth of penetration was not consistent. In addition, the number of tubules containing bacteria varied from tooth to tooth and among sections of an individual tooth. The different depths of penetration may be related to the bacterial size, its adhesive, and its motility characteristics but also to differences in the lumen diameters that are attributed to natural spatial variations or caused by occlusion of the tubules related to age. The degree of permeability varies between different areas of a tooth and the number of patent dentinal tubules present (11). Bacteria can invade these dentinal tubules, and bacterial products can diffuse across dentin to elicit pulpal reactions (12, 13). The penetration of bacteria in dentinal tubules could be a determinant of their virulence in the root canal environment. It was also reported that the cervical and midroot areas were penetrated by Streptococcus gordonii to a depth of 200 μm, whereas invasion of the apical dentin was significantly lower to a depth of 60 μm (14). The difference could be attributed to the lower number of patent tubules in the apical region because of dentinal sclerosis, which is always more advanced in this region than coronal and midroot dentin at any age (15). Indeed, the tubule diameter plays an important role on the depth of bacterial invasion (16). Tubules that are sclerotic or obliterated can physically impede bacterial invasion and result in regional differences in bacterial invasion of dentin.

Dentin undergoes an increase in mineral content with age caused by filling of the tubule lumens (17) and, in some instances, total obliteration of the tubules takes place (18). In a scanning electron microscopic examination of human dentin in teeth from patients ranging from 20 to above 80 years of age, the number of dentinal tubules decreased significantly (p < 0.001) with increasing age and apical location (19). It was hypothesized that the increased calcification of the apical dentin in seniors may help prevent periapical irritation, and the apical dentin of teeth of young people would have more dentinal tubules with a greater chance to harbor potentially irritating bacteria. Therefore, the aim of the present study was to determine if age affects the number of infected dentinal tubules and the depth of bacterial penetration inside the dentinal tubules.

Materials and Methods

Fifty-six extracted single-rooted human maxillary or mandibular teeth were obtained from the oral surgery clinic at the University of Maryland Dental School according to a protocol approved by the Institutional Review Board. Only intact virgin teeth extracted because of orthodontic treatment or periodontal disease were included. The teeth were divided equally into young and old groups, where the mean ages of the patients were 22.1 (range, 18–24) and 67 (range, 60–77) years old, respectively.

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The teeth were then sterilized using ethylene oxide to reduce the chance that their physical and biological properties may be inadvertently affected by autoclaving. An access opening through the crown was made using a size 4 round bur, and canals were instrumented to a size 40 Profile 0.06 taper file (Tulsa Dental, Dentsply, Tulsa, OK). The instrumentation was used to standardize the size of the canals, to ensure a consistent volume of bacterial inoculum, and to remove soft tissues. Irrigation with 5.25% NaOCl (10 mL) and 17% EDTA (3-mL final rinse) was performed. Then, a 5% solution of sodium thiosulfate was used for irrigation and as a medium for overnight storage in order to neutralize the effects of NaOCl.

Twenty-six teeth in each group were considered the experimental teeth, and the remaining two teeth per group were used as controls. The experimental teeth were placed in an anaerobic chamber, and the canal space was filled with about 10 to 15 μL of thioglycolate culture fluid, which contained 3 × 10^8 cells/mL of Enterococcus faecalis (ATCC 19433). This facultative anaerobic bacterial species was selected because it is present in all phases of the development of an infection in root canals (20–22), and it can efficiently and rapidly colonize the tubules in comparison with other strains.

All experimental samples were incubated for 20 days at 37°C. Reinoculation was performed at the end of the first week and the second week, according to methods previously described by Saleh et al. (23). After the completion of reinoculation, access openings were closed using OptiBond Solo adhesive resin (Kerr Corp., Orange, CA) and TPH composite caulk (Dentsply, Millford, DE) in order to prevent any contamination of the root canals during further processing. Two control teeth in each group were not inoculated.

**Histological Analysis**

Histological analyses of all the specimens were performed to determine the degree of infection within the dentinal tubules. Samples were fixed in 4% paraformaldehyde overnight and then demineralized in 17% EDTA and agitated constantly for 75 days. EDTA was used because it is less likely to destroy the bacterial cells to where they are not visible than strong acids. The specimens were paraffin-embedded and processed for light microscopic examination. Four, 5-μm sections were obtained at both 2 mm and 6 mm from the apex for each root. Two samples in the young group and one sample in the old group were lost during the sectioning. Therefore, a total of 24 and 25 samples in the young and the old groups, respectively, were observed under light microscopic examination.

Each section was stained with Brown and Brenn’s bacterial stain for light microscopic examination (24). Two magnifications (200 and 1,000) were used for observation. All samples were observed with a 1,000× magnification to count the total number of tubules invaded by bacteria and a 200× magnification to measure the depth of bacterial penetration inside tubules from the canal side. The total number of tubules invaded by bacteria was counted and averaged in the four sections of specimens at both 2 mm and 6 mm from the apex in the young and the old groups. Each slide was positioned on the microscope stage in such a way that a maximum of 20 tubules were visible in the entire observation field. At this point, counting of the number of the invaded tubules was made. The depth of bacterial penetration from the canal was determined by measuring the deepest penetration within any tubule in the field to the closest 25 μm at either the 2-mm or 6-mm section.

**Figure 1.** The depth of bacterial invasion in a section of (A) young dentin and (B) old dentin. Note that more tubules in the young dentin stained positive for bacteria (Brown and Brenn; 1,000× magnification).

**Figure 2.** (A) The mean number of dentinal tubules invaded by bacteria in the young and the old groups in a field of 20 tubules (F = 6.25, p = 0.014). (B) The mean depth of bacterial invasion in the young and the old group (F = 4.82, p = 0.033).
Data Analysis

A power analysis was performed a priori to determine sample size. This analysis showed that using 26 young and 26 old teeth with an effect size of 0.40 (a relatively large effect size) resulted in a power of 0.81, which was an acceptable statistical power. One-way analysis of variance was used to compare results for the young and old teeth; p < 0.05 was considered significant.

Results

Most specimens showed the penetration of dentinal tubules by bacteria. A representative cross-section of bacterial penetration within the tubules at the 6-mm level is shown for the young and old dentin in Figure 1. Out of 24 and 25 samples in the young and the old group, one sample in the young group and two samples in the old group did not show penetration of bacteria in the four sections of specimens at 2 mm and 6 mm from the apex. Also, the two controls in each group did not show bacteria at any level.

Results of the tubular invasion measurements were analyzed to determine differences associated with patient age. In the 2-mm sections, bacteria invaded a higher number of dentinal tubules in the young group than the old group, although the difference was not significant (p = 0.09). A similar trend was observed in the 6-mm sections, and again the difference was not statistically significant (p = 0.862). Nevertheless, when results of the 2-mm and 6-mm sections were combined (Fig. 2), a higher number of invaded tubules by bacteria were observed in the young group (p = 0.014).

The mean depth of bacterial invasion in the young and the old group was approximately 420 μm and 360 μm, respectively (Fig. 2). Bacteria penetrated the young radicular dentin to a significantly deeper level than the old dentin (p = 0.053). There were no significant differences observed between the 2-mm and 6-mm sections in either group.

Discussion

This study examined the degree of penetration of bacteria into dentinal tubules of root canal walls in young and old teeth. A higher number of infected dentinal tubules and deeper bacterial penetration were found in the young compared with the old teeth. This may be explained by the presence of more sclerotic dentinal tubules in the old dentin at any level of the root. A difference in the number of infected dentinal tubules may be clinically relevant because it may impact the ability of dentin to harbor bacteria after endodontic infection and the ability of clinical procedures to disinfect the canal wall.

With regard to location, no significant differences were observed between infected tubules at the 6-mm and the 2-mm levels. It was previously noted that the increased formation of peritubular dentin, which leads to a decreased number of open dentinal tubules, occurs more rapidly in the apical region and with advancing age and that these differences may have clinical implications (19). Our findings do not corroborate these earlier observations.

The results of this study may offer an explanation for why some studies have shown that older individuals have a higher success rate in endodontic therapy (25, 26). In an endodontic treatment outcome cohort study, a significantly higher success rate of endodontic treatment was shown in patients older than 60 years of age when compared with two younger groups (40–60 and less than 40 years) (25). It may be that in older patients, less residual bacteria in dentinal tubules result in a lower bacterial load and thus reduced periapical radiolucency. Orstavik et al. (26) speculated that the progressive reduction of pulp space, diversities, and ramifications with age limits the volume available for infection and makes it easier to provide adequate canal debridement and root filling.

The present study showed a significant difference in the depth of bacterial penetration inside the dentinal tubules between the two age groups. On average, bacteria invaded old dentin 65 μm less than the young dentin. These findings are consistent with those from an earlier study in which the maturity of the tooth was a major factor in tubule bacterial penetration (6).

Despite the potential importance of the findings to endodontic practice, there were recognized limitations to the investigation that should be addressed. Periodontally involved teeth were used, primarily in the older group, and this may have affected the results. We are not aware of published data on tubule occlusion in periodontally diseased versus nondiseased teeth. This experiment also used only one type of bacteria to evaluate the number of infected tubules or the depth of penetration inside dentinal tubules. Because endodontic infections are polymicrobial in nature, these results may not be relevant to other bacterial species that have less ability to penetrate dentinal tubules.

The results of this study clearly revealed the influence of age on tubular infection. It can be speculated that the higher number of tubules invaded by bacteria and the greater depth of penetration in the young group could account for increased periapical irritation and delayed healing in this age group. Future studies should explore whether there are differences in the ability of clinical procedures to disinfect dentinal tubules in young and old teeth.

References


