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# Dentin Permeability of the Apical Third in Different Groups of Teeth

Rodrigo Gonçalves RIBEIRO[1]
Melissa Andreia MARCHESAN[2]
Ricardo Gariba SILVA[1]
Manoel D. SOUSA-NETO[1]
Jesus Djalma PÉCORA[1]

[1]*Department of Restorative Dentistry, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto, SP, Brazil*
[2]*Nova Southeastern University, College of Dental Medicine, Fort Lauderdale, FL, USA*

This *ex vivo* study evaluated dentin permeability of the root canal in the apical third of different human groups of teeth. Eighty teeth were used, 8 from each dental group: maxillary and mandibular central incisors, lateral incisors and canines, maxillary first premolars (buccal and palatal roots), mandibular first premolars, and maxillary and mandibular second premolars, totalizing 88 roots that were distributed in 11 groups. The root canals were instrumented, irrigated with 1% NaOCl and 15% EDTA. Roots were immersed in 10% copper sulfate for 30 min and then in 1% rubeanic acid alcohol solution for the same period; this chemical reaction reveals dentin permeability by the formation of copper rubeanate, which is a dark-colored compound. Semi-serial 100-µm-thick cross-sections were obtained from the apical third of the roots. Five sections of each apical third were washed, dehydrated, cleared and mounted on glass slides for examination under optical microscopy. The percentage of copper ion infiltration and the amount of tubular dentin were quantified by morphometric analysis. The penetration of copper ions in the apical third ranged from 4.60 to 16.66%. The mandibular central and lateral incisors presented the highest dentin permeability (16.66%), while the maxillary canines and mandibular second and first premolars presented the lowest dentin permeability (4.60%, 4.80% and 5.71%, respectively; $p<0.001$). The other teeth presented intermediate permeability. In conclusion, dye penetration into dentin tubules at the apical region is strongly dependent on the group of teeth evaluated.

Key Words: dentin permeability, root canal, dental groups.

## INTRODUCTION

Different methodologies have been used to evaluate the root canal dentin permeability before and after the use of irrigating solutions, i.e., radioisotopes (1), histochemical method (2,3), and dyes (4,5).

The variations in dentin microstructure, its chemical composition, the formation of dentin tubules, the deposition of the mineralized matrix during dentinogenesis, the inherent permeability of dentin, and the different types of dentin have been extensively investigated (5-10). However, there are still issues not totally understood or that still need discussion. Dentin permeability can be influenced by physiologic changes that lead to the formation of sclerotic dentin (4-6).

Penetration of microorganisms into dentin tubules can be influenced by the type of dentin. Kakoli et al. (10) showed deeper microbial penetration into the dentin of young teeth (420 µm) compared to elderly teeth (360 µm).

Based on the fact that the apical third of the root canal is less permeable than the middle and cervical thirds (2,11,12), this *ex vivo* evaluated the apical permeability of the different groups of teeth and the pattern of distribution of tubular and sclerotic dentin.

## MATERIAL AND METHODS

Eighty *ex vivo* teeth were selected, 8 from each dental group (maxillary and mandibular central incisors, lateral incisors, canines, 1st and 2nd premolars). Because the first premolar presents 2 roots, this study used 88 roots that were divided into 11 groups. The teeth were radiographed and examined under stereoscopic magnification to select only those with completely formed roots, a single canal, and absence of calcification or resorptions were used.

Teeth were rendered water-proof with a

Correspondence: Dr. Melissa A. Marchesan, 3200 South University Drive, Fort Lauderdale, FL, USA. Fax: +1-954-262-1782. e-mail: meljul@uol.com.br

cyanoacrylate adhesive (Super-Bonder; Henkel Loctite Adesivos Ltda., Itapevi, SP, Brazil). Access to the pulp chambers was done considering the anatomical aspects of each group of teeth. A #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) was introduced into each canal until it appeared at the apical foramen. The working length was established by subtracting 0.5 mm from this length.

Mechanical preparation was performed using the K3 system (Sybro Eendo, Orange, CA, USA), activated by an electric motor X-Smart (Dentsply-Maillefer) at 350 rpm. The cervical third was prepared with a #2 LA Axxess bur (#35/.06, Sybron Endo) and the root canal was prepared with a #20/.02, 20/.04, 25/.02, 25/.04, 30/.02, 30/.04, 35/.02, 40/.02 and 45/.02 instruments. During instrumentation, the teeth were irrigated with 2 mL of 1% NaOCl between files, followed by irrigation with 15% EDTA for 3 min, and final irrigation with 10 mL of distilled and deionized water to remove chemical residues.

### *Histochemical Preparation*

The teeth were then immersed in 10% copper sulfate aqueous solution (Merck, Darmstadt, Germany) for 30 min, in a vacuum for the first 5 min, dried with paper points (Dentsply-Maillefer) and placed in 1% rubeanic acid alcohol solution (Merck) for 30 min, in a vacuum for the first 5 min. Rubeanic acid reveals copper ions, forming a compound ranging in color from deep blue to black, depending on the quantity of copper ions present. Teeth were kept in closed jars with ammonium-embedded cotton pellets for color fixation. Upon completion of this reaction, each tooth was placed in an acrylic resin block and 100-µm-thick transverse sections were obtained with a diamond disk from the apical 4 mm (representing the apical region). During the sectioning process, constant irrigation with water was used to prevent dentin burn.

The slices were then sanded under tap water to a thickness of approximately 70 µm and washed for 4 h, dehydrated in a series of increasing alcohol solutions (70, 80, 96, twice in 100% alcohol) for 2 h each, cleared 3 times in xylol, and mounted on glass slides for microscopic examination.

Quantification of the penetration of copper ions was done by morphometric analysis with a 400-point grid. The percentage of copper ion penetration into dentin was calculated by the following equation: $Pd\% = PS/PT \times 100$, where PS = number of points in the stained area and PT = total number of points in stained and non-stained areas of dentin (Fig. 1). During this process, tubular distribution (tubular and sclerotic dentin) was also evaluated. Data were subjected to Tukey's HSD multiple-comparison test for statistical analysis at a 0.05 significance level (17.0 SPSS Program for Windows, Chicago, IL, USA).

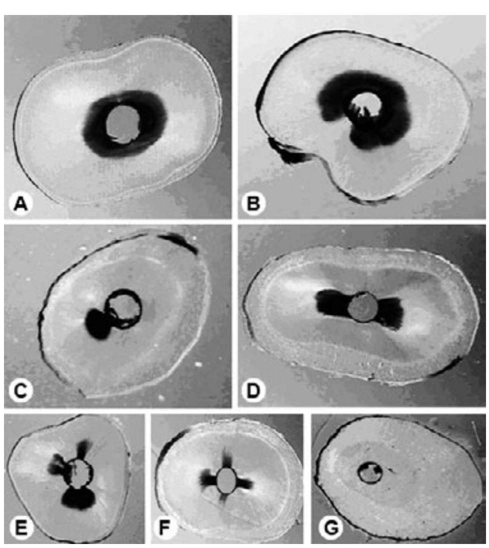


Figure 1. Patterns of copper ion penetration into the tubules surrounding the root canal lumen: A = Type I: total distribution around the canal; B = Type II: semi-total distribution; C = Type III: unipolar distribution; D = Type IV: bipolar distribution; E = Type V: tripolar distribution; F = Type VI: tetrapolar distribution; G = Type VII: no penetration (total presence of sclerotic dentin).

## RESULTS

ANOVA showed a significant difference in copper ion penetration into dentin among the different groups of teeth ($p=0.0000$). The Tukey's HSD test was used to identify these differences and the data are shown in Table 1.

When reviewing the photographs, it was evident that dye penetration into the dentinal tubules was not equally distributed. The qualitative distribution of the

Table 1. Percentage of copper ion penetration (mean ± SD) into apical dentin of the different groups of teeth .

| | |
|---|---|
| Mandibular central incisor | 16.66 ± 2.23 ♣ |
| Mandibular lateral incisor | 16.66 ± 5.35 ♣ |
| Maxillary 1st PM - palatal root | 13.42 ± 6.85 ♥ ♣ |
| Max. 1st PM - buccal root | 12.37 ± 5.19 ♥ ♣ |
| Mandibular canine | 10.77 ± 2.39 ● ♥ ♣ |
| Maxillary 2nd premolar | 8.08 ± 2.79 ● ♥ |
| Maxillary lateral incisor | 7.67 ± 4.38 ● ♥ |
| Maxillary central incisor | 6.70 ± 4.44 ◊ ● |
| Mandibular 1st premolar | 5.71 ± 4.84 ◊ |
| Mandibular 2nd premolar | 4.80 ± 3.45 ◊ |
| Maxillary canine | 4.60 ± 1.92 ◊ |

Same symbol shows no statistically significant difference ($p>0.05$).

apical permeability (presence of tubular and sclerotic dentin) allowed us to classify the permeability into 7 types as shown in Table 2. This classification corresponds to the double effect, which indicates at the same time the presence of dentinal tubules and absence of sclerotic dentin. Figure 1 illustrates the shapes of the tubular distribution and the presence of sclerotic dentin found in the apical third of the studied groups of teeth.

## DISCUSSION

Permeability is an inherent characteristic of dentin due to the presence of tubules. This property varies according to the quantity and diameter of the tubules of each region of the root canal walls (7,13,14). Other factors can also influence permeability such as external aggression caused by pathological processes or tooth aging (5,6,10).

The transparent dentin (sclerotic) shows the changes in the diameter of the tubules with different degrees of lumen occlusion due to the apposition of peritubular dentin associated with the deposition of calcium salts in the intrabulular dentin (6,9). The sclerotic dentin appears permanently at the mesial and distal walls of the canals and, with age, surrounds the entire root (15). Kusunoki et al. (13) concluded that the sclerotic dentin is effective for adhesion in the crown. Kwong et al. (16) found that micromechanical retention alone may be hindered by the sporadic absence of the hybrid layer and resin tags in sclerotic dentin. Interestingly, Tay and Pashley (17) reported that resin bonds to sclerotic dentin can be extended to include peripheral sound dentin in order to increase bond strength without need of additional retentions to the crown.

The histochemical method uses the smaller molecule size of copper ions compared to organic dyes, which are insoluble in xylol allowing clearing of dentin without staining (2,3). It is important to acknowledge that since the publication of Pashley's study in 1978 (18), it is known that the size of the molecule is fundamental when detecting dentin permeability. Those authors reported that a 19-fold increase in molecule size would decrease permeability 100 times. It is also important to acknowledge that Feigl (19) reported that rubeanic acid reveals copper ions forming a stained compound ranging in color from deep blue to black depending on the quantity of copper ions present, with a limit for identification of copper at 0.006 µg, showing the sensitivity of the method.

In the present study, dye penetration into dentin tubules at the apical region was strongly dependent on the group of teeth evaluated. Maxillary canines (4.66%) and mandibular first (5.71%) and second (4.80%) premolars presented the lowest dentin permeability, while the mandibular central and lateral incisors presented the highest permeability. This may be because maxillary canines, and mandibular first and second premolars suffer great impact during mastication compared to the mandibular central and lateral incisors (10).

The maxillary central and lateral incisors, second maxillary premolars and mandibular canines presented intermediate permeability, and were sometimes similar to the groups of teeth with greater permeability and sometimes similar to the groups of teeth with lower permeability. These differences may be due to age, attrition, abrasion or other types of stress (8,10,20).

The palatal and buccal roots of the maxillary first premolars presented statistically similar permeability in the apical third probably due to the fact that these teeth underwent the same stress throughout life.

To explain the low permeability of the apical dentin of the maxillary canines, first and second mandibular premolars, one should consider the fact

Table 2. Classification of the distribution of dentin tubules in the apical third of root canals.

| Type of tooth | Type I | Type II | Type III | Type IV | Type V | Type VI | Type VII |
|---|---|---|---|---|---|---|---|
| Max. central incisor | 4 (10.0%) | 1 (2.5%) | 19 (47.5%) | 6 (15.0%) | 1 (2.5%) | 0 | 9 (22.5%) |
| Max. lateral incisor | 5 (12.5%) | 2 (5.0%) | 7 (17.5%) | 4 (10.0%) | 2 (5.0%) | 13 (32.5%) | 7 (17.5%) |
| Max. canine | 0 | 1 (2.5%) | 12 (30.0%) | 5 (12.5%) | 3 (7.5%) | 0 | 19 (47.5%) |
| Max. 1st premolar-BR | 2 (5.0%) | 15 (37.5%) | 14 (35.0%) | 5 (12.5%) | 0 | 0 | 4 (10.0%) |
| Max 1st premolar-PR | 11 (27.5%) | 9 (22.5%) | 6 (15.0%) | 11 (27.5%) | 0 | 0 | 3 (7.5%) |
| Max 2nd premolar | 3 (7.5%) | 0 | 4 (10.0%) | 28 (70.0%) | 1 (2.5%) | 0 | 4 (10.0%) |
| Mand. central incisor | 0 | 4 (10.0%) | 6 (15.0%) | 15 (37.5%) | 6 (15.0%) | 7 (17.5%) | 2 (5.0%) |
| Mand. lateral incisor | 0 | 5 (12.5%) | 6 (15.0%) | 17 (42.5%) | 10 (25.0%) | 2 (5.0%) | 0 |
| Mand. canine | 0 | 1 (2.5%) | 10 (25.0%) | 23 (57.5%) | 0 | 0 | 6 (15.0%) |
| Mand. 1st premolar | 1 (2.5%) | 4 (10.0%) | 6 (15.0%) | 16 (40.0%) | 1 (2.5%) | 0 | 12 (30.0%) |
| Mand. 2nd premolar | 6 (15.0%) | 5 (12.5%) | 5 (12.5%) | 7 (17.5%) | 2 (5.0%) | 2 (5.0%) | 13 (32.5%) |

Data are reported as number with percent in parenthesis. A total of 40 slices were evaluated for each group of teeth. BR: buccal root; PR: palatal root.

that these teeth present a higher incidence of sclerotic dentin (Type VII: maxillary canines, 47.5%; first premolar, 30%; second premolar, 32.5%). Also based on the proposed classification, the mandibular central and lateral incisors showed a high pattern of types II, III, IV and V sclerotic dentin, and a low incidence of type VII.

These results confirm the findings of Mjör et al. (8) and Thaler et al. (5) on the irregularity of the dentin structure at the apical region of root canals. The presence of sclerotic dentin in all root canal walls of the apical third (type VII) and the sclerotic dentin between the dentinal tubules (unipolar, bipolar, tripolar, tetrapolar and semitotal) are probably caused by the aggression these teeth undergo while in use in the oral cavity: caries, attrition, abrasion, occlusal trauma, among others, which can activate the defense process of the dentin-pulp complex and obliterate the dentin tubules, and lead to the formation of translucent or sclerotic dentin especially in the apical third (5,9,10). Thaler et al. (5) reported results that demonstrate that dentinal dye penetration was strongly dependent on the location of the root canal section and decreased from coronal to apical.

Based on the presence of sclerotic, transparent and occluded dentin in the apical third of root canals, some teeth are not the ideal specimens for microbiological studies since the absence of bacteria in the dentinal tubules could be due to the lack of penetration rather than the effect of an antibacterial agent. This should also be taken into account when performing apical leakage studies as well as endodontic treatment procedures.

## RESUMO

Este estudo *ex vivo* avaliou a permeabilidade da dentina do canal radicular do terço apical de diferentes grupos de dentes humanos. Foram utilizados 80 dentes, sendo 8 de cada grupo dental superior e inferior: incisivos centrais, incisivos laterais, caninos, primeiros pré-molares superiores (raízes vestibulares e palatinas), primeiros pré-molares inferiores, segundos pré-molares superiores e inferiores, totalizando 88 raízes, distribuídas em 11 grupos. Os canais foram instrumentados, irrigados com NaOCl a 1% e EDTA a 15%. As raízes foram imersas em sulfato de cobre a 10% por 30 min e acido rubeânico a 1% pelo mesmo período. Esta reação química revela a permeabilidade da dentina por meio da formação de um complexo escurecido denominado rubeanato de cobre. Hemi-secções de 100 µm de espessura foram obtidas do terço apical da raiz. Cinco secções do terço apical foram lavadas, desidratadas, diafanizadas e montadas em lâminas para análise em microscopia óptica. A porcentagem de infiltração de íons cobre e a quantidade de dentina tubular foram quantificadas por meio de análise morfométrica. A penetração de íons cobre no terço apical da raiz variou de 4,60 a 16,66%. Os incisivos centrais e laterais apresentaram a maior permeabilidade dentinária (16,66%), e os caninos superiores e segundos e primeiros pré-molares inferiores as menores (4,60, 4,80 e 5,71%, respectivamente; $p<0,001$). Os outros dentes apresentaram permeabilidade intermediaria. Conclui-se que a penetração de corante nos túbulos dentinários da região apical é extremamente dependente do grupo de dentes avaliado.

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