# Evaluation of accessory furcation canals of permanent mandibular molars using radiography and clearing

## Avaliação do canal cavo-interradicular em molares inferiores permanentes através de Rx e diafanização


### Abstract

Purpose: To evaluate the presence of furcation canals of permanent mandibular teeth using radiography and a clearing technique.

Methods: The sample comprised 344 extracted mandibular molars. The presence of furcation canals was assessed by a single trained observer using magnifying lens (4x) for the dental radiographs and a dental optical microscope (30x) for the cleared specimens. *Scanning electron microscopy* (SEM) was used to evaluate morphological differences in the pulp chamber floor.

Results: Radiographs showed that 9% of the specimens had radiolucent areas, 2% had an image that suggested a canal, and 89% had no abnormal findings. Clearing techniques did not show any accessory canal. SEM images revealed dentin tubules in recently extracted teeth; the other specimens had small areas with dentin tubules.

Conclusion: Radiography was not better than the clearing technique to diagnose furcation canals. The clearing technique can provide three-dimensional visualization of the internal tooth anatomy for *in vitro* studies.


**Key words:** Furcation defects; anatomy; histology; tooth demineralization; radiography; molars


### Resumo

Objetivo: Avaliar a presença do canal cavo-interradicular em molares inferiores permanentes através de radiografias e diafanização.

Metodologia: A amostra foi constituída por 344 molares inferiores re-hidratados, preparados e armazenados individualmente em recipientes de vidro. A presença do canal cavo-interradicular foi investigada por um único operador treinado usando lupa (4x) para as radiografias e microscópio óptico odontológico (30x) para as amostras diafanizadas. *Microscopia eletrônica de varredura* (MEV) foi usada para verificar diferenças morfológicas do assoalho pulpar.

Resultados: A análise radiográfica mostrou que 9% das amostras tinham uma zona levemente radiolúcida, 2% mostravam uma imagem sugestiva, e 89% das amostras não tinham nenhuma evidência. Pela diafanização, o canal não foi encontrado nas amostras avaliadas. Pela MEV, as amostras recém extraídas mostraram com canalículos dentinários uniformes; as demais apresentaram pequenos sítios com canalículos uniformes.

Conclusão: O exame radiográfico não foi o melhor método de diagnóstico; a diafanização é um excelente método avaliativo, pois permite a visualização tridimensional da anatomia interna dental em pesquisas *in vitro*.


**Palavras-chave:** Defeitos na furca; anatomia; histologia; dentes desmineralizados; radiografia; molares


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## Introduction

The success of endodontic treatment depends on sound knowledge of internal dental anatomy because of the presence of accessory, lateral, secondary and furcation canals. Accessory furcation canals cross the dentin between roots in permanent (1-5) and primary (3) molars and connect the pulp chamber to the periodontal ligament in the furcation region (2,5-8). They are a path for contamination from the pulp to the periodontium (or vice-versa), which explains the relapse of periodontal and/or endodontic lesions after endodontic treatment and bone loss in the furcation area (3,4,6,9-13).

Previous studies showed the existence of accessory canals in the furcation region and the pulp chamber floor using different methods for permanent mandibular molars, but the prevalence rates are very different (1,4-8,10,12,14-17). These results may reflect variations related to sample size as most studies included about 55 specimens (1-3,5-7,10,12,15,17). Only a few studies employed larger samples: 100 to 200 (4,16), 300 to 400 (8), or 859 teeth (14). The origin of accessory canals, particularly accessory furcation canals, is a failure in the formation of the Hertwig's sheath (3,6,18), which Figun and Garino (18) called a physiological periodontal fistula through which arterioles, pulp and collagen fibers can grow (3).

Most studies that used radiography evaluation adopted techniques similar to those used in clinical practice (1,5, 8,10,14,15,17). However, other authors have added unusual projections (14,15,17) to obtain detailed results using radiographs. To visualize all the extension of the accessory furcation canal and confirm its presence, a clearing technique may be used to render the specimen transparency and make the canal visible (5). Clearing is a simple and inexpensive technique that provides three-dimensional visualization of teeth and preserves the original form of the root canal system (5,16,17,19,20). There is still a need to establish a gold standard technique to detect accessory, lateral, secondary and furcation canals.

This study evaluated the presence of an accessory furcation canal in 344 permanent human mandibular molars comparing radiography and a clearing technique.

## Methods

This study was approved by the Committee for Ethics in Research of the School of Dentistry, Federal University of Santa Maria – UFSM, Santa Maria, RS, Brazil.

The sample comprised 344 human permanent mandibular molars from the tooth bank of the Dental Anatomy Course of the Morphology Department of our institution. The sample included human permanent mandibular teeth that were intact or had occlusal caries or restorations but intact pulp chamber, with the furcation clearly exposed, i.e., the roots were separated in the furcation region, but roots should be either complete or incomplete. Exclusion criteria were: abnormal pulp chamber floor or cervical third of the crown; fused roots; not clearly visible furcation (the roots were too close to each other).

### Sample preparation

As the teeth had been completely dehydrated for storage, it was necessary to rehydrate them to avoid fracture during preparation. The specimens were immersed in distilled water in a covered container and kept at a temperature of 37 ± 1 °C for 14 days; the water was changed every 2 days. Afterwards, the specimens were stored in distilled water under refrigeration to maintain hydration (21).

During preparation, two cuts were made in each specimen using a # 4102 diamond bur in a high-speed handpiece under constant water-spray irrigation. The bur was replaced after the preparation of each group of 20 specimens. The first cut was made at 1.5 mm from the furcation apically, and the second, at the cementoenamel junction on the buccal surface. After that, the second cut was made cervically until 0.5 mm from the pulp chamber floor. The canals were slightly enlarged using a #20 endodontic file and a three-way air/water syringe.

The samples were immersed in 1% sodium hypochlorite solution for 24 h (5) to dissolve the tissues on the dentin surface (10), rinsed under running water and immersed in 1% sodium hypochlorite in an ultrasonic bath for 10 min. They were then rinsed under running water, dried at room temperature (±20ºC), and stored individually in glass containers closed with silicone stops (Fig. 1).


Fig. 1. Glass containers with labeled specimens.

### Radiographic examination

All specimens were radiographed using a 50 kVp X-ray unit (Trophy Radiologie, Marne la Vallee, France) with X-ray film (AGFA Dentus M2, E/F speed, Heraeus Kulzer, South Bend, IN, USA) in a periapical film holder (Indusbello, Londrina, PR, Brazil). Four specimens were radiographed on each piece of film using a standard radiographic projection

and a 7.5 cm work distance. Specimens were fixed to the holder with utility wax, and the holder was fixed to the X-ray cone with adhesive tape. After that, the specimen was exposed for 1 s.

All radiographs were immersed in a developing solution (Kodak, Rochester, NY, USA) for 2 min, rinsed with water for 20 s, and immersed in fixing solution (Kodak, Rochester, NY, USA) for 10 minutes. After that, they were immersed in water for 5 min and left to dry at room temperature for 24 h (22). A 4x magnifying lens and an X-ray box were used for the evaluation of the images by a single trained examiner.

## Clearing

Specimens were immersed in 5% hydrochloric acid solution for 72 h (5), and the solution was changed every 24 h (4,5). The specimens were rinsed under running water for 10 min and immersed in distilled water for 6 h; the water was changed every hour (4). The specimens were dehydrated following an increasing series of ethanol concentration: 80% for 12 h, 90% for 2 h and 100% ethanol for 2 h (4).

Immediately after that, the specimens were left to dry at room temperature, and about 0.5 mL India ink was injected through the coronal chamber using an insulin syringe. The specimens were immediately immersed in absolute ethanol for 1 h to fix the dye to the dental structure (5). After fixation, all specimens were immersed in methyl salicylate (4,5,17,19,20) for about 2 h to achieve complete transparency (4) and kept in this solution to ensure that they remained clear (Fig. 2).

The canal system of each specimen was analyzed using a dental microscope (Model DM 2003, 30x magnification, Opto Eletrônica, São Carlos, SP, Brazil) and placing the specimen in the glass container on an X-ray viewer box . The images were recorded using a digital camera (Nikon Coolpix 950, Nikon Corporation, Tokio, Japan) coupled to the microscope.

All specimens were evaluated by the same trained observer, and data were recorded in an Excel spreadsheet for descriptive statistical analysis.

## Scanning Electron Microscopy

Scanning electron microscopy (SEM) (Jeol Inc. Tec., Tokyo, Japan) was used to perform a qualitative analysis and to compare the morphological characteristics of the dentin in the pulp chamber floor of the dehydrated teeth used in this study with the dentin of recently extracted teeth. Six specimens that were not included in the sample were prepared by using a # 4102 diamond bur in a high-speed handpiece under water irrigation until the pulp chamber floor was visible; the cervical wall of the pulp chamber was drilled with the same bur. Two of these specimens were extracted molars stored for no longer than six months in saline solution, and four were stored in dry containers in the tooth bank of the Dental Anatomy Course; two were rehydrated as described above.

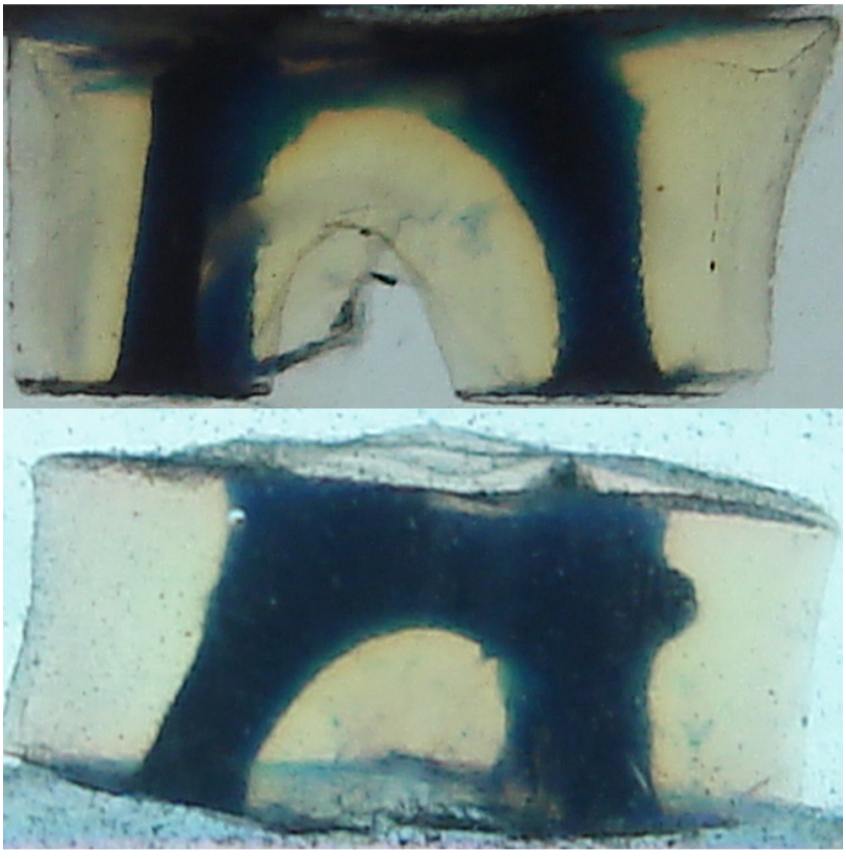

**Fig. 2.** Cleared specimens.

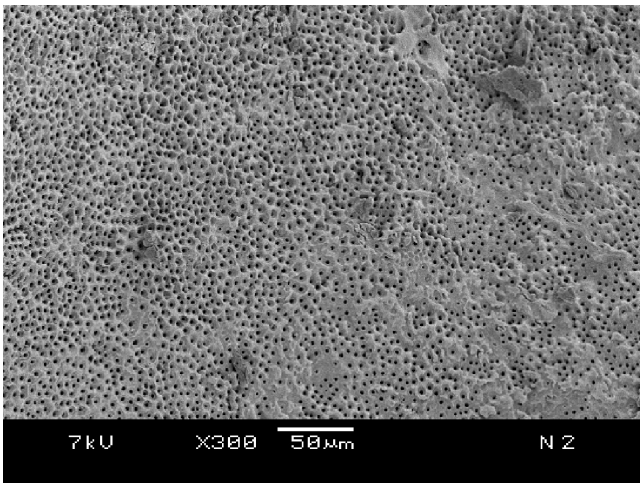


**Fig. 3.** Normal samples under *scanning electron microscopy* (300x).

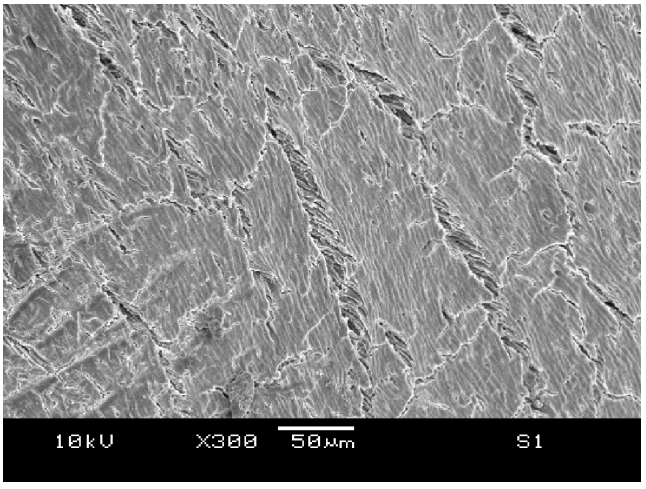


**Fig. 4.** Dry samples under *scanning electron microscopy* (300x).

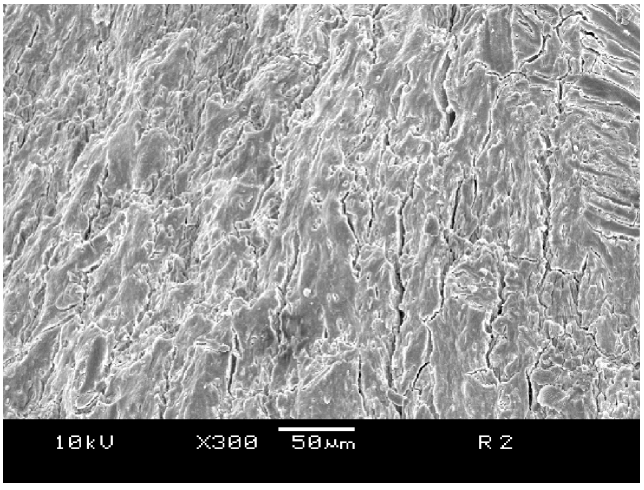


**Fig. 5.** Rehydrated specimen under *scanning electron microscopy* (300x).

The specimens were immersed in 1% sodium hypochlorite solution for 24 h (5), rinsed under running water and immersed in 1% sodium hypochlorite in an ultrasonic bath for 10 min. They were then rinsed under running water, dried at room temperature (±20º), and the specimens were prepared and stored individually in small glass containers labeled with an adhesive tag containing the following identification in capital letters: S1 and S2 (dry, dehydrated specimens); Re1 and Re2 (rehydrated specimens); and N1 and N2 (extracted molars, that is, normal specimens).

For SEM, the specimens were immersed in 2.5% glutaraldehyde in 0.1 M buffered sodium cacodylate (6 h) and dehydrated through a series of ethanol baths at 60%, 70%, 80% and 90% (5 min each) and 3 more hours in 100% ethanol. After that, the specimens were removed from the solution, left to dry at room temperature (± 20ºC) for some seconds, wrapped in gauze individually, and stored in sealed surgical packages for 24 h until submitted to gold sputtering (Desk II, Denton Vacuum, Moorestown, NJ, USA). Images were obtained using the SEM software at 300x magnification (Fig. 3, 4, 5).

## Results

Table 1 shows the percentages of radiographic findings. Radiographs showed that 9% of the specimens had radiolucent areas, 2% had an image that suggested a canal, and 89% had no abnormal findings. Clearing techniques did not show any accessory canal. SEM images revealed dentin tubules in recently extracted teeth; the other specimens had small areas with dentin tubules.

**Table 1.** Frequency (absolute and percentage) of specimens with findings that suggested the presence of an accessory furcation canal according to the radiographic evaluation.

| Accessory furcation canal | n | % |
|---|---|---|
| 0 | 305 | 89.6 |
| Possible canal | 8 | 2.3 |
| Furcation with slight radiolucency | 31 | 9.1 |

## Discussion

The presence of furcation canals (2,4,5,8,16,17) or accessory canals in the furcation area (3,6,7,10,12) are well-documented and be the cause of recurrence of endodontic problems or periodontitis in the furcation area (3,4,6,9-13). Furcation canals are found in maxillary or mandibular molars, both in the permanent (2-5) and primary (3) dentition. This study investigated their presence in permanent mandibular molars because their anatomy provides clearer radiographic images than primary teeth.

Radiography can be used in the clinics or in the laboratory to study dental internal anatomy. In this study, radiographs were taken using one projection and only one direction (1, 5,10), but no radiopaque material was injected into the pulp cavity (5,10).

A suggestive image of a furcation canal was found in eight specimens (2.3%). This percentage is slightly lower than the 3.5% found by Almeida et al. (5), but higher than the null frequency found by Vertucci and Anthony (10) and Motta and Milano (1).

In 9% of the specimens, the radiographs did not show the accessory canal as a clear image, but as a slightly radiolucent area in the furcation region. This percentage and the 2.3% of presence of furcation canal were not confirmed using the clearing technique, which did not show any furcation canals. Almeida et al. (5) also found that radiograph images may only suggest the presence of a furcation canal, but the clearing technique may confirm its presence. Conversely, some authors detected furcation canals (4,5,16).

The present study showed that the radiographic images suggested the presence of a canal or of slightly radiolucent structures in the furcation region, but they were shown not to be canals when the clearing technique was used. The study by Motta and Milano (1) did not find any accessory furcation canals using radiographs and radiopaque endodontic cement in 19 mandibular molars. However, Almeida et al. (5) examined 29 mandibular molars and found one canal in one sample using radiography, and one canal in two samples when using a clearing technique.

Clearing is achieved in three stages: decalcification, dehydration and clearing itself (4,5,16,17,19,20). This procedure is exclusively performed for research in the laboratory, being impossible to be used in clinical practice. The technique can be used to confirm the existence of furcation canals in study specimens because it is a simple technique that provides three-dimensional visualization of the canal system (5,17,19,20).

The clearing technique has some limitations, such as the lack of uniform clearing in some samples. This may be associated with the duration of the decalcification (demineralization) stage, when dental enamel is dissolved in hydrochloric acid and only demineralized dentin is left. The greater the specimen weight, the longer the immersion in acid should be (19). All specimens in this study were immersed for 72 h, although they had different volumes and, consequently, different weights. Therefore, variation in dental weight may be responsible for the variation in the clearing findings.

Another limitation of this clearing technique is the procedure to determine the exact moment to stop dental decalcification. Some suggest the use of a needle to transfix the specimen (4) or to hear for a rubberlike sound (19). The latter was chosen for the present study; although subjective, the procedure is more conservative, and the specimen's structure was not affected. As the specimens were placed in glass containers individually, the container was energetically shaken every time the acid solution was changed, and the sound produced could be easily heard: if low and buffered, the specimen should have a rubberlike consistency and be totally decalcified, which was confirmed to occur at 72 hours according to a pilot test.

The pulp cavity can only be seen when the specimen is cleared if it is filled with some endodontic filling material or dye, such as hematoxylin (16), vinyl polysiloxane (19) or India ink (4,5,17,19,20). Methylene blue cannot be used when clearing is performed with methyl salicylate because it is dissolved in contact with it (23).

The use of India ink was chosen because of the size of most particles, which measured 3.79 to 5.88 µm (24), values that were smaller or very close to the small diameters of the foramina in the pulp chamber floor, which ranged from 4 to 260 µm when visualized using SEM (3,10), as well as in the furcation, ranging from 4 to 437 µm (3,10). Dye particles can penetrate the accessory furcation canal, which is classified as a foramen. Moreover, this dye cannot penetrate the dentin tubules because their diameters range from 0.6 to 3 µm which, therefore, does not confirm that endodontic-periodontic contact occurs through these tubules.

Future studies using clearing techniques should seal the apical foramina with wax before the beginning of the process and the decalcification of the specimens to avoid staining the external surface of the tooth, which is totally dehydrated and avid for humidity during dye injection into the pulp cavity. This type of stain was seen in some of the specimens in this study.

In the same procedure, during the dye fixation in ethanol, the amount of India ink may be smaller than the amount of about 0.5 mL used per sample in this study. This change may reduce the staining of the external specimen surface. Staining does not make visualization impossible, but the specimen surface is cleaner if it is avoided.

No accessory furcation canal was detected using the clearing technique, which raised the hypothesis that the original tooth storage of completely dehydrated teeth might have affected the results. Macroscopically, it was not possible to see any anatomic differences (naked eye). SEM was used for six specimens to investigate whether there was any difference on the dentin surface of the pulp chamber floor. In the dehydrated and rehydrated specimens, the dentin surface seemed to be covered with small plaques, and a small number of dentinal tubules was visualized when compared with the sample of recently-extracted teeth.

In the study by Fawzy (25), specimens were demineralized using 37% phosphoric acid, followed by deproteination with 6.5% sodium hypochlorite for 120 s, and the images obtained were evaluated under atomic force microscopy after 3, 6, 9 and 12 minutes of exposure to air. After image evaluations, the authors suggested that air exposure for 12 minutes resulted in dentinal dehydration and collapse of collagen fibers, which, due to lack of peripheral humidity, blocked dentinal tubules and superficial foramina. Therefore, those images were similar to the ones in this study (Figures 4 and 5), which supports the hypothesis that dehydration may be responsible for the surface changes observed.

## Conclusions

Under the conditions of this study, radiography was not a superior diagnostic method to visualize accessory furcation canals, but it was a good complementary test and important

for the planning of clinical procedures. Clearing is an excellent assessment method because it provides full three-dimensional visualization of the internal dental anatomy, and is an easy and inexpensive technique. Further studies should be conducted to evaluate the use of dehydrated teeth in different study methods.

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