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Assessment of Morphological Changes on the Pulpal Wall of Root Dentine During Growth

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Abstract: The aim of this study, is to evaluate the changes appearing on the pulpal wall of root dentine with age with special reference to the number and diameter of dentinal tubules' openings as well as to the morphology of calcospherites. For this purpose 38 permanent human teeth, 22 premolars and 16 canines, of patients between 9 and 60 years of age, were experimentally fractured. After specific preparation they were studied with Scanning Electron Microscope (SEM). It was assessed that the number and width of dentinal tubules was considerably greater in teeth of younger patients and the highest number of openings and greatest diameter were observed at sides were formation was ongoing. The pulpal surface of dentine of younger patients was relatively smooth and after the removal of organic material and predentin an active front of calcification was observed by means of calcospherites. In teeth of older patients, the pulpal surface of dentine was irregular and instead of an active calcification process, a calcified net of interlacing branches of collagen fibers was observed. Lateral root canals were seen in a greater amount in younger patients. The calcification process inside and around the lateral root canals was intense compared to adjacent sides showing formation of predentine and active front calcification. These findings indicate the significant changes appearing with age on the pulpal surface of root dentine implying changes in dentine permeability.

Key words: Active calcification process, calcospherites, dentinal tubules, SEM

INTRODUCTION

The superior characteristics of the second hardest substance of human teeth, the dentine, are definitely dentinal tubules, providing elastic support to the enamel (Elbaum *et al*., 2007). They are tube-like channels of 1-2 μm diameter radiating from the enamel-dentine and cementum-dentine junctures to the pulp and are related to the presence of odontoblasts' prolongations during deposition and mineralization of the organic substrate of dentine (Porter *et al*., 2005). The communication between the pulp chamber and root canal with the external environment may lead to inflammations of the pulp and periodontal ligament damages (Harran Ponce *et al*., 2001). The lumen of the tubules changes in diameter, particularly decreasing from the pulp to the enamel or cementum. Changes occur also with age because of the formation of peritubular dentine that reduces the diameter gradually to their external opening. Altered forms of dentine may also occur due to physiological changes or the formation of carries. In these cases, a mineral substance having a refractive index similar to that of dentine, probably of salivary origin, occludes the channels and leads to the formation of the so-called sclerotic or translucent dentine with changes in the number of tubular openings (Porter *et al*., 2005; Whittaker and Bakri, 1996; Sengupta *et al*., 1999). In case of dental, caries sclerotic dentine is assessed at the cervical part of root dentine. In contrast physiological stimuli lead to the formation of sclerotic dentine starting from the apical part of the root dentine (Whittaker and Bakri, 1996).

Methods used to study the microstructure of dentine underwent changes during the last decades. In particular, optical microscopy was replaced from electron microscopy and techniques using fluorescent labeling and even computer tomography (Elbaum *et al*., 2007). In the present study scanning electron microscopy was used in order to assess the surface topography of root dentine.

MATERIALS AND METHODS

In the present study, 38 teeth of the permanent dentition, 22 premolars and 16 canines, where prepared and studied with scanning electron microscope. This

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study was carried out, at the department of Endodontics during the past 3 years. As mentioned by former scientists the use of prepared surfaces render SEM examination difficult. In particular, the remaining scratches on the surfaces examined interfere with the discernment of the samples. On the other hand, fractured surfaces are more suitable for the observation. In our study, the teeth were fractured and etched with sodium hypochlorite (undiluted, for at least 24 h) in order to remove the non-mineralized matrix and for a better observation of the predentine-dentine junction.

The samples for SEM examination were obtained after fracturing the samples leading to a stepped emergence of tubular openings with some of them appearing transversely or longitudinally and some obliquely. In order, to achieve the desired fracture a blade was inserted in preformed cavities on the tooth surface and the blade was used as a lever. Pulp remnants were removed taking care of the pulpal surface of the dentine.

In order to obtain, the original position of the samples after fracturing, they were assembled so that they reform the original section. In particular, the fractured samples of the whole teeth were mount on aluminum stubs with Durafix.

After this initial preparation, the mounted samples were placed in a coating machine under vacuum for at least 24 h to remove reagents and vapor. Finally, coats of carbon and gold of 0.015 μm thickness were applied to overcome the electrical non-conductivity of the teeth.

Serial SEM photomicrographs at various magnifications were taken from the pulpal wall of dentine at the apex.

RESULTS AND DISCUSSION

SEM examination revealed that the numerical density of the openings of dentinal tubules on the pulpal wall of dentine was greater in the teeth of younger compared to older individuals, especially at sides of ongoing formation (Fig. 1a-f).

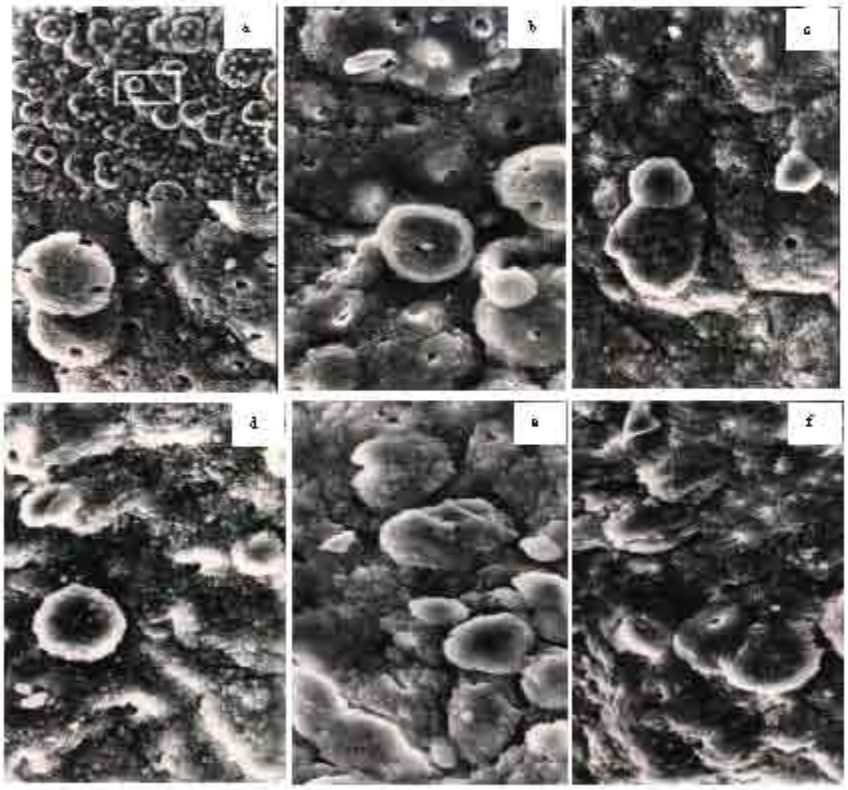

Fig. 1: a): The surface of the pulpal wall of root dentine in a young individual after the preparation with NaOCl (x400 magnification). b: Calcospherites of the deep layer are fused but the superficial calcospherites remain nummular (x2000, young subject). c, d): Calcospherites of the deep layer are fused and the numerical density of superficial calcospherites and tubular openings is lesser in middle-aged individuals (x2000) e, f): In older individuals calcospherites of the deep-layer are completely fused and the superficial calcospherites are irregular in shape. No openings of dentinal tubules were observed

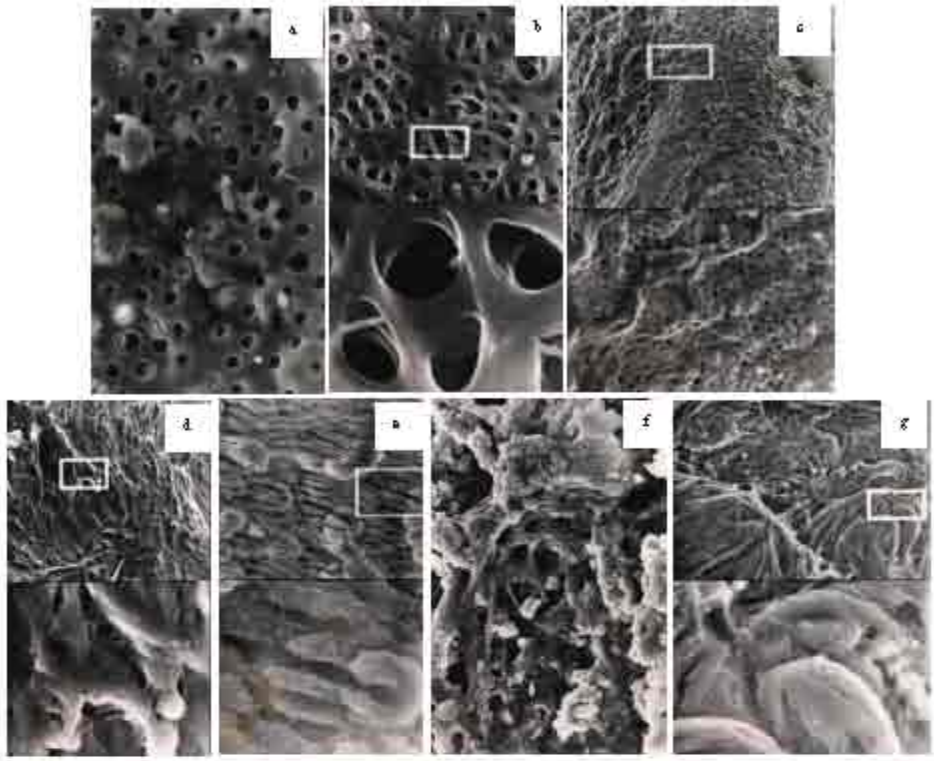

Fig. 2: Smooth dentine surface with the characteristics of predentine in a sample of a young individual (a, x2000 magnification, b, x1500). Irregularities of the pulpal wall of dentine in a middle-aged individual (c, x150). Profound irregularities in older individuals (d, x1000). Layers of calcospherites with divergent degrees of fusion and a dentinal tubule following through its course in a young individual (e, x800, NaOC1 etched). Irregularities of the pulpal wall of dentine in a middle-aged and older individuals after NaOC1 etching (f, x2000 and g, x1000 particularly)

The pulpal wall of root dentine was in teeth of younger individuals' relative smooth. After the preparation with NaOC1 that enables the removal of organic remnants and the observation of the samples, predentine was seen to present an active front of mineralization by means of calcospherites. In contrast, samples of older individuals revealed an irregular surface of the pulpal wall of root dentine and the calcospherites in predentine were fused, presenting a calcified net of interlacing branches of collagen fibers (Fig. 2a-g).

Lateral root canals were seen in a greater amount in younger patients (Fig. 3a-e).

An active calcification process was observed inside and around these root canals compared to older individuals. This front of mineralization was characterized by a layer of predentine with a higher rate of calcification compared to adjacent sides (Fig. 4a-d).

Many new approaches for the study and observation of root dentine were developed in order to assess morphological changes of root dentine's pulpal wall during growth. The use of SEM is of particular value because it enables the observation of the real morphology of the micro-structure of a tooth without disturbing its natural condition and characteristics. In addition, this technique enables large samples to be observed by means of whole teeth fractured by hand having a high resolution potential compared to classical optical microscopy.

New approaches such as the 3 dimensional reconstruction of images taken with pulsed infrared laser enable the observation of the 3D structure of dentinal tubules and the collagen fibrils around them (Elbaum *et al*., 2007). In particular the use of these new techniques affords the opportunity to study the interface between dentine and artificial materials, to distinguish secondary from primary dentine, to detect bacterial infection and crack formation. It is clear that the study of root dentine morphology and its changes during growth can be enhanced with the use of new imaging techniques

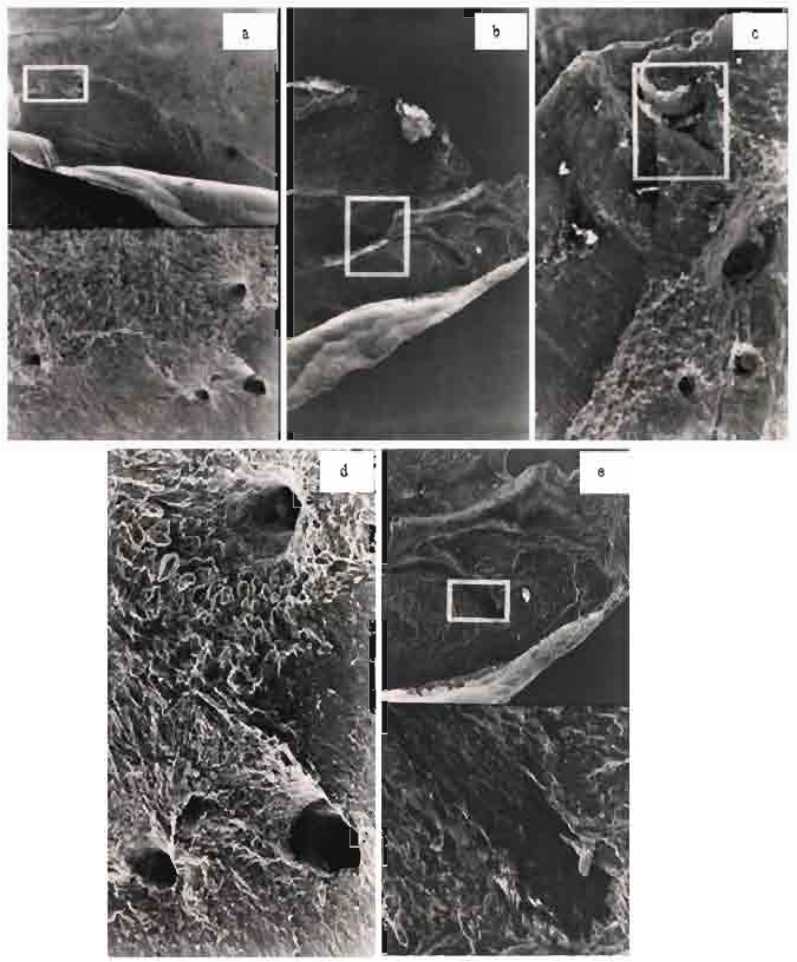

Fig. 3: Overall aspect of the root with ongoing formation of the apical area. Openings of 4 lateral root canals can be seen (a, x25, young individual, NaOCl etched). Opening and course of a lateral root canal (b, x30, old individual, NaOCl etched). The three openings of the lateral root canals observed in the former photograph as well as the external opening of a lateral root canal (c, x100, young individual, NaOCl etched). In c a magnification of the former photograph shows the openings of the lateral root canals. Details of the opening and course of the lateral root canal and cementum of the apex (d, x50, NaOCl etched)

and that the combination of scanning electron microscope and 3D imaging techniques may lead to qualitatively better observations.

It is well established that dentine undergoes morphological changes during growth, with transparency representing the most common age-related change. The detection of the transparent zones in dentine may support age-determination and the evaluation of the effect of this process on tooth resistance (Porter *et al*., 2005). Except this process the formation of secondary dentine and/or fibrodentine leads to changes in the dental pulp and to a restriction mostly in a mesiodistal dimension more often present after the 6th decade of life (Schroeder, 1993).

The present study provides, conclusive evidence that age-related chances appear on root dentine and lead to a decrease in the number and diameter of openings of dentinal tubules on the pulpal wall of root dentine. In addition it is advocated that the number of openings decreases in a corono-apical direction (Schilke *et al*., 2000). These changes are related to the previously mentioned by many scientists process of translucency formation. It starts from the apex at the zone of the dentine next to the cementum and gradually reaches the cervical area of the tooth spreading towards the pulpal wall. The observed translucent zones represent occluded dentinal tubules and former investigations have proven evidence of the fact that the occluding material is hydroxyapatite in larger crystals compared to the surrounding intertubular dentine, due to the resolution of crystals that belong to the intertubular dentine (Porter *et al*., 2005). It is

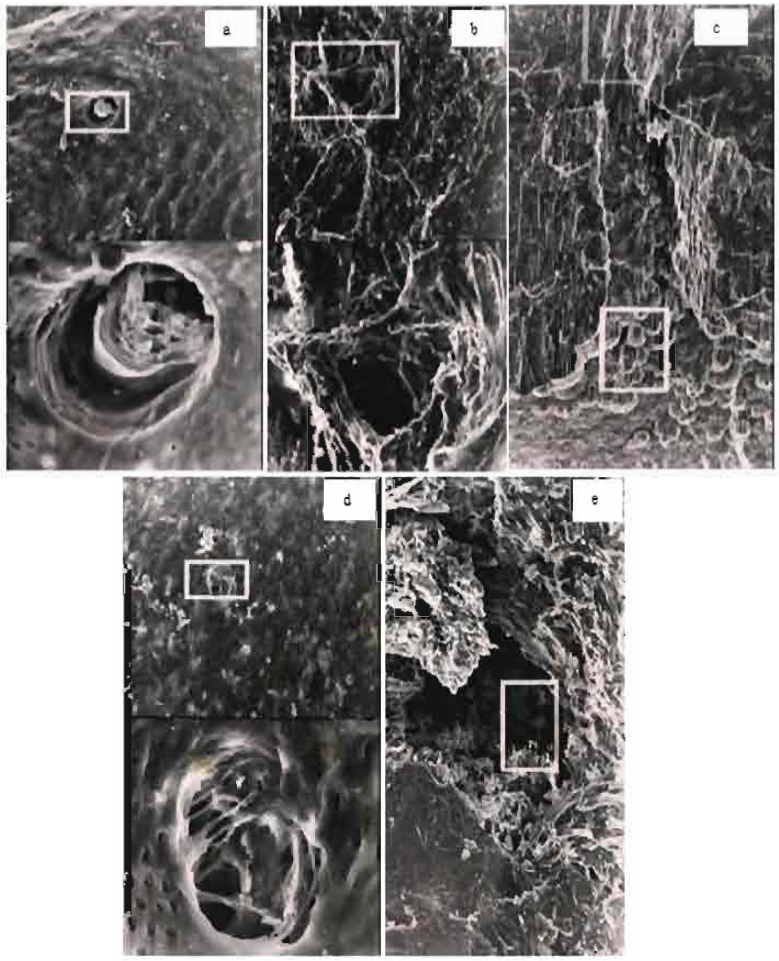

Fig. 4: a) The opening of a lateral root canal with signs of profound calcification inside and around the opening in a young individual (x200). Opening of a lateral root canal with organic components interiorly (b, x250, old individual). Opening and course of a lateral root canal. High numerical density of calcospherites implies profound calcification (c, x350, young individual, NaOCl etched). Ongoing calcification was observed in a middle-aged individual inside and around a lateral root canal (d, x250). Organic content was assessed in a sample of a young patient after NaOCl etching (e, x400)

advocated that the process is a passive procedure and it is not clear whether there are other changes in dentine (Kinney *et al*., 2005). In addition, scientists phrase the hypothesis that the difference in the concentration of minerals decreases towards the apex. This is probably due to the decrease in density of tubular openings (Kinney *et al*., 2005). The hyper-mineralization observed in translucent dentine is probably the cause for the absence of a normal predentine layer observed in our present study and mentioned in previous surveys. It must be mentioned that this change in morphology is more often assessed in non-vital teeth compared to vital ones (Sengupta *et al*., 1999).

This study offers, interesting findings on how age leads to microstructural changes in dentine that affect tooth resistance and enhance tooth fractures.

The age-related feature of root dentine associated with the formation of translucent zones can be etiologically associated with the reduction of stimuli transduction to human periodontium during maturation, with the physiological mineralization of intratubular dentin or due to toxins produced in the diseased periodontium (Whittaker and Bakri, 1996). No differences regarding age were seen but the correlation of root dentine translucency and age seems to be affected by racial diversities, which are in close relation to environmental factors and diet.

Finally, the potential of the area of translucency to help estimating the age is of higher value if compared to the length but the possibility of post-depositional changes during preparation must be taken into account and further studies must be carried out in order to assess the factors that have an effect on root dentine. Additionally, the presence of changes in dentinal features alone can not support the age-estimation because these changes can be the sign of an early tooth wear as mentioned by Hojo (1990), in his study that refers to Japanese population.

The present study advocates that the dentine undergoes morphological changes during growth, leading to a decreased density of tubular openings on the pulpal wall of root dentine, a phenomenon associated with the formation of translucent dentine starting from the apex of a tooth, the formation of secondary dentine or/and fibrodentine. The clinical importance of this finding is that these teeth are probably more prone to fractions, a possibility increasing gradually with age. This may explain the increased number of fractions of endodontically treated teeth, a hypothesis that is less related to dentine dehydration or alteration of the mineralization of intertubular matrix, as mentioned in previous surveys (Kinney *et al*., 2005).

Secondary dentine may be part of a physiological change of root dentine during growth. As assessed in our study an irregular surface was observed with SEM examination associated with an irregular area of calcification, compressed calcospherites and a convoluted arrangement of dentinal tubules. It is worth, noting that the changes appeared on the mid and lower root level and not on the upper level where the calcospherites are formed semiglobularly (Wakabayashi *et al*., 1983).

CONCLUSION

The present survey achieved to assess with the use of scanning electron microscope the significant changes appearing with age on the pulpal surface of root dentine implying changes in dentine permeability. These changes are dominated by a decreased number and diameter of tubular openings on the pulpal wall of root dentine, a diminished extent of active calcification and irregularities of the surface of root dentine. In addition, a decreased number of lateral root canals were observed.

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REFERENCES

Elbaum, R., E. Tal, A.l. Perets, D. Oron, D. Ziskind and Y. Silberberg *et al*., 2007. Dentin micro-architecture using harmonic generation microscopy. J. Dent., 2: 150-155.

Harrán Ponce, E., C. Canalda Sahli and J.A. Vilar Fernandez, 2001. Study of dentinal tubule architecture of permanent upper premolars: evaluation by SEM. Aust. Endod. J., 2: 66-72.

Hojo, T., 1990. Scanning electron microscopic quantitative study of changes with age in closing pattern of openings of dentinal tubules on worn occlusal surfaces of Japanese permanent mandibular incisors. Scanning. Microsci., 4: 1049-1053.

Kinney, J.H., R.K. Nalla, J.A. Pople, T.M. Breunig and R.O. Ritchie, 2005. Age-related transparent root dentin: mineral concentration, crystallite size and mechanical properties. Biomaterials, 16: 3363-3376.

Porter, A.E., R.K. Nalla, A. Minor, J.R. Jinschek, C. Kisielowski and V. Radmilovic *et al*., 2005. A transmission electron microscopy study of mineralization in age-induced transparent dentin. Biomaterials, 36: 7650-7660.

Schilke, R., J.A. Lisson, O. Bauss and W. Geurtsen, 2000. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch. Oral. Biol., 5: 355-361.

Schroeder, H.E., 1993. Age-related changes in the pulp chamber and its wall in human canine teeth. Schweiz. Monatsschr. Zahnmed., 2: 141-149.

Sengupta, A., D.K. Whittaker and R.P. Shellis, 1999. Difficulties in estimating age using root dentine translucency in human teeth of varying antiquity. Arch. Oral. Biol., 11: 889-99.

Wakabayashi, H., K. Matsumoto, Y. Nakamura and T. Shirasuka, 1983. Morphology of the root canal wall and arrangement of underlying dentinal tubules. Int. Endod. J., 3: 153-8.

Whittaker, D.K. and M.M. Bakri, 1996. Racial variations in the extent of tooth root translucency in ageing individuals. Arch. Oral. Biol., 41 (1): 15-9.