SEM Morphology of Peritubular and Intertubular Root Dentin of Young, Adult and Senescent Teeth

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Abstract: ***Dentinis considered as a peculiar tissue regarding new insights about its composition. Dentine is composed of tubules surrounded by a hyper-mineralized layer called peritubular dentin, and a softer intertubular matrix.Its organic components concentrates in form of collagen fibrils and non-collagenous proteins and complex macro-molecules.The aim of this study is to analyze the morphology of peritubular and intertubular dentin of young, adult and senescent teeth in the coronal, middle and apical sections of the root. Permanent human third molars (n=30) were examined by SEM. Our study is in parallel to the recently proposed model of peritubular dentin that provides novel evidence about structure and chemistry of peritubular dentin. It lacks collagen fibrils, contains an organic scaffold embedded with mineral and the peritubular dentin organic matrix is manly composed of glycosaminoglycans, whereas the lamina limitans is primarily made of proteoglycans protein cores. Age-related characteristics of root dentin are evident in peritubular meshwork thickening, reduction of inter-fibrillar connections and mineral deposition within the tubules.***

Keywords: peritubular dentin, age-related characteristics of dentin, SEM, lamina limitans

1. Introduction

Dentinconstitutes the bulk of the tooth and is considered as a peculiar tissue regarding new insights about its composition, especially non-collagenous proteins and additional macromolecules with complex chemistry and origin.From a physiological point of view, dentin can be described as a nanostructured vital semi-permeable barrier between enamel and pulp [1], whereas biomechanically it functions as a tougher foundation helping to prevent propagation of cracks from the brittle enamel [2].

2. Literature Survey

Dentine is composed of tubules, measuring about 1–2 µm diameter surrounded by a hypermineralized layer called peritubular dentin, and a softer intertubular matrix, where the organic material concentrates in form of collagen fibrils and noncollagenous proteins, such proteoglycans, glycosaminoglycans and phosphorilated proteins.

3. Problem Definition

The aim of this study is to analyze the morphology of peritubular and intertubular dentin of young, adult and senescent teeth in the coronal, middle and apical sections of the root.

4. Methodology/Approach

Material – sample selection and preparation

Permanent human third molars (n=30) were obtained according to protocols approved by the Ethical Committee of Medical University – Plovdiv, Bulgaria. All teeth were fixed in neutral formalin solution (10%) for 24-72 hours and assembled in three groups: young root dentin (in 18-35 years old patients), adult root dentin (in 36-50 years old patients) and senescent root dentin (above 51 years old patients). The specimens were dehydrated in ascendinggrades of ethanol and acetone (from 25% to 100%). Fresh fractured fragments were subsequently coated with gold for 10 min in vacuum.

Method

The images were gained using a SEM (JSM-IT500HR, USA).

5. Results and discussion

The steps involved in the formation of the peritubular dentin are not well understood. Recent studies have suggested that it may form from an extracellular matrix rich in glutamic acid [3, 4, 5], or by continuous mineral deposition from the pulpal fluid, which results in the accumulation of mineral around the tubules [6].

Our results show expected cuff-like structure of the peritubular mineral surrounding the tubules (Fig.1) in young root (apical area) dentine specimens. The intertubular matrixis characterized by the roughened collagen-rich area whereas the mineral-rich peritubular dentin appears as a smoother structure.

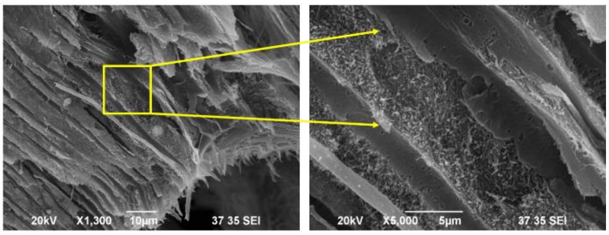

Figure 1: Root (apical area) young dentine specimen

Normal dentin showing the expected well defined tubular structure within the intertubular matrix and the peritubular mineral cuff surrounding the tubules. The intertubular matrix is characterized by the roughened collagen-rich area whereas and the mineral-rich peritubular dentin is appears smoother. Root (apical area) young dentine.

A thin membrane apparently covering the visible length of the tubule walls with the expected sheet-like pattern previously described as the lamina limitans [7], or outer fibrillar organic sheath [8], is visible (Fig. 2).

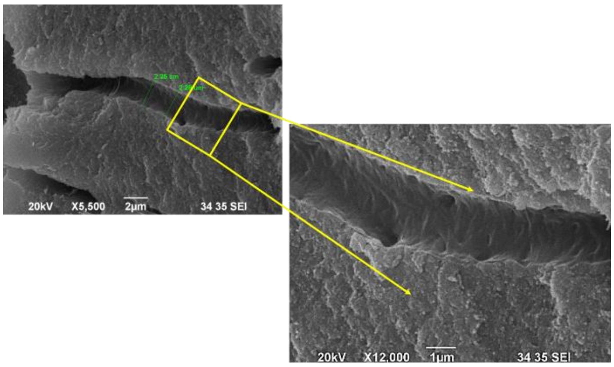

Figure 2: Fig. 9 Root (coronal area) adult dentine specimen

The tubule walls with the expected sheet-like pattern previously described as the lamina limitans.

Early microscopy studies suggested that the peritubular dentin contained an organic sheath delineating its inner and outerlimits [9, 10, 11, 12, 13]. Studies on partially demineralized dentin also revealed fine organic fibrils [12, 13], a flatwork of delicate fibrils [14], and a low density of amorphous substance [11] by TEM and SEM.

Further, our results illustrated that in the region initially occupied by the peritubular mineral, well defined filaments that appeared to be organic in nature became evident in young coronal root dentin as well as in adult specimens (Fig. 3, 4, 5)

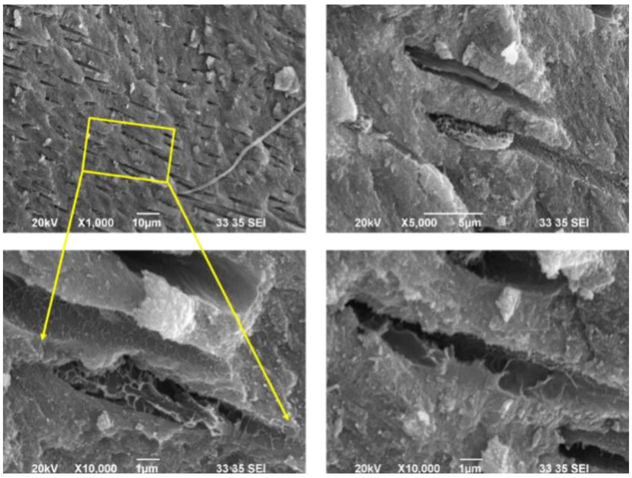

Figure 3: Root (coronal area) young dentine specimen

Complex organic meshwork in peritubular dentin, filaments interconnections.

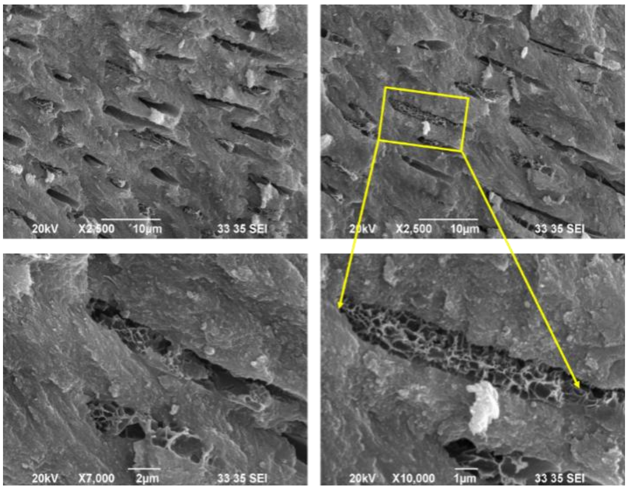

Figure 4: Root (coronal area) young dentine specimen

Complex organic meshwork in peritubular dentin, filaments interconnections.

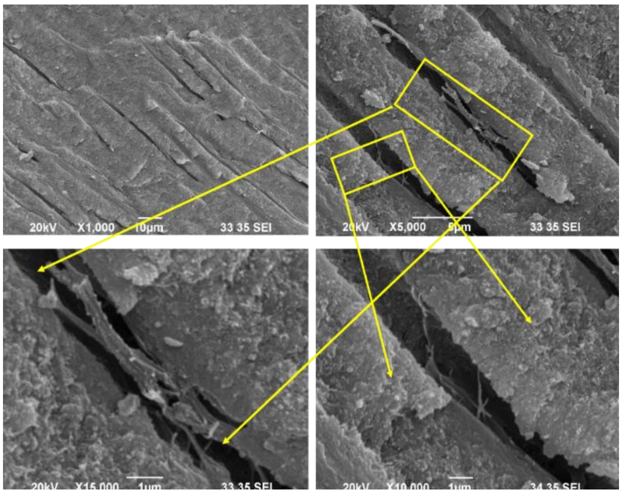

Figure 5: Root (coronal area) adult dentine specimen. The organicfilaments protrude from the tubule walls

Careful observation reveals that these organicfilaments protrude from the tubule walls, thus indicating that they originate from the lamina limitans and extend towards the tubule lumen (Fig. 6).

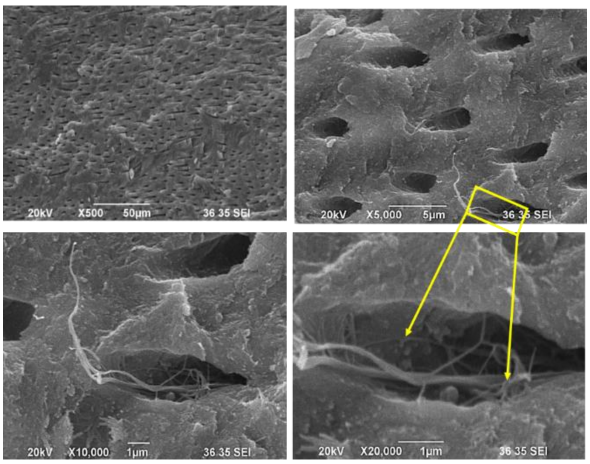

Figure 6: Root (apical area) adult dentine specimen. The organicfilaments protrude from the tubule walls, and the meshwork is interconnected.

A difference in the orientation of the organic fibrils is generally unclear, although they are described previously as semi-concentric fibrils and rod-shaped radial fibrils in peritubular dentin from horse [8].At ultra-high magnification (Fig. 6, x20 000) it is evident that the complex meshwork of organic filaments is interconnected by unidentified structure, which we speculate could be phosphorilated matrix proteins, lipids or other matrix macromolecules,or structural artefacts that resulted from accumulation of by-products of the demineralization treatment[15].

This indicated that the peritubular dentin organic matrix may be a complex of phosphorilated proteins, proteoglycans and glycosaminoglycans, lacking collagen fibrils [16]. Proteoglycans, as a biological entity, are composed of a protein core [17] and carbohydrate component that is called glycosaminoglycan. It forms the primary element responsible for the structural stability of the extracellular matrix of nearly all vertebrates [18, 19]. In dentin, the most prominently expressed proteoglycan protein cores are decorin and biglycan[20], whereas the mostly frequently found glycosaminoglycans are chondroitin 4-sulfate and a relatively lower content of chondroitin 6-sulfate, although dermatan sulfate, hyluronan and keratan sulfate have also been reported [20]. The intratubular filaments are also apparent in senescent specimens. The meshwork consists of thicker filaments as well as simplified interconnections (Fig.8) and mineral deposits within the dentinal tubules (Fig.9).

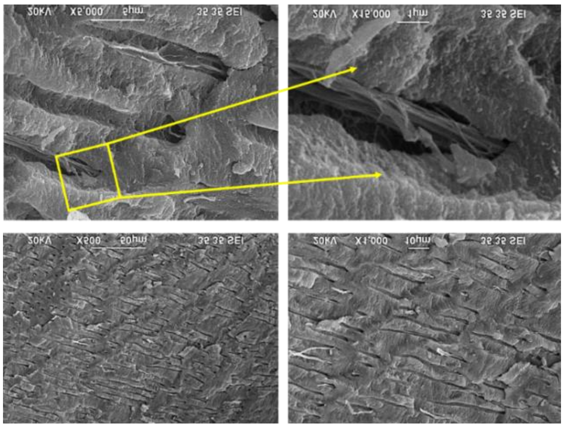

Figure 7: Root (middle area) senescent dentine specimen. Thicker organic meshwork in peritubular dentin, simplified filaments interconnections.

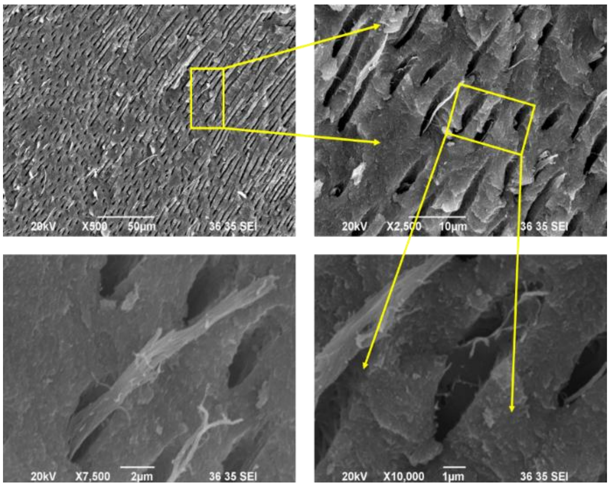

Figure 8: Root (middle area) senescent dentine specimens

Thicker organic meshwork in peritubular dentin, simplified filaments interconnections.

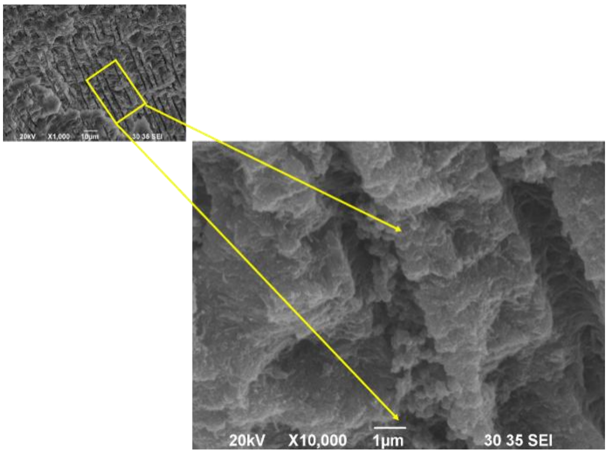

Figure 9: Root (apical area) senescent dentine specimens. Mineral deposits within the dentinal tubules

Our results are also consistent with findings reported by Luiz et al. [15], Orgel et al. [21], Gotlivand Veis[3, 4]. All authors hypothesize that the peritubular matrix may be organized as a complex system whereby proteoglycans attach to collagen fibrils [21] at the tubule walls, glycosaminoglycans side chains extend and protrude towards the tubule lumen interconnectingwith each other forming a scaffold embedded and coated with mineral binding proteins as well as other attached matrix macromolecules, such as phospho-lipid–proteolipid complexes [16], which then recruit mineral precursors initiating mineralization and may also participate in biological signaling mechanisms.

6. Conclusion

Our study is in parallel to the recently proposed model of peritubular dentin structure by Luiz et al. [15] who provide novel evidence about structure and chemistry of peritubular dentin. It lacks collagen fibrils, contains an organic scaffold embedded with mineral and the peritubular dentin organic matrix is manly composed of glycosaminoglycans, whereas the lamina limitans is primarily made of proteoglycans protein cores. Age-related characteristics of root dentin are evident in peritubular meshwork thickening, reduction of inter-fibrillar connections and mineral deposition within the tubules.

7. Future Scope

We acknowledge the limitations of our study regarding the lack of selective enzymatic disintegration or digestion of non-collagenous proteins "building" the peritubular dentin as well as the evaluation of intratubular mineral deposits in aged root dentin. The examination of the described structures on molecular level may enable the verification of the proposed theories in our study. These research work may give new perspectives an understanding of the human dentin and related diagnostic, therapeutic and preventive strategies.

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Author Profile

Neshka Manchorova, Graduated from the Medical University – Plovdiv, Bulgaria in 2000. Started working as an assistant professor in 2002, associate professor in 2012 and professor in 2016 in the Department of Operative Dentistry and Endodontics in the same university. Successfully defended her PhD thesis in May 2009 on the topic of “Postoperative sensitivity in posterior composite restorations”. Areas of interest include the restoration of endodontically treated teeth, biomechanical properties of dentin, adhesive strategies, and molecular biology.