



## ASSESSMENT OF SECONDARY DENTINOGENESIS ON PERMANENT MOLARS – A MICRO-CT STUDY

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### SUMMARY

**Purpose:** This study quantitatively evaluates secondary dentinogenesis in permanent molars by comparing the volume and thickness of enamel, coronal dentin, and pulp chamber in immature and mature teeth.

**Material and methods:** Forty-eight extracted third molars were analyzed using micro-computed tomography (micro-CT). The sample included 24 mature (from patients aged 25-30) and 24 immature teeth (from patients aged 15-18), all without caries lesions or other defects. Morphometric measurements assessed the volumes of enamel, dentin, and the pulp chamber, as well as the thickness of enamel and dentin in the area of the mesiobuccal (MB) cusp and central pit.

**Results:** The results revealed no significant difference in enamel volume or thickness between the two groups. However, mature teeth exhibited a significantly greater coronal dentin volume ( $309.884 \pm 42.982 \text{ mm}^3$  vs.  $250.560 \pm 47.025 \text{ mm}^3$ ,  $p = 0.001$ ) as well as increased thickness of the dentin at the MB cusp ( $p = 0.003$ ) and central pit ( $p = 0.001$ ). Additionally, the pulp chamber volume was significantly reduced in mature teeth ( $13.712 \pm 3.443 \text{ mm}^3$  vs.  $19.759 \pm 4.883 \text{ mm}^3$ ,  $p < 0.001$ ).

**Conclusion:** These findings highlight the progressive deposition of secondary dentin with age, which reduces the size of the pulp chamber and increases dentin thickness, particularly in high-risk areas like the MB cusp. This study underscores the importance of understanding structural differences between immature and mature molars for informed clinical decision-making in caries management and minimally invasive dentistry.

**Keywords:** Secondary dentinogenesis, Permanent molars, Immature teeth, Micro-CT, Enamel volume, Dentin volume, Pulp chamber,

### INTRODUCTION

Mature permanent teeth are characterized by several features: they have completely formed roots with a closed root apex, thicker and more mineralized enamel and dentin, a smaller pulp chamber volume due to the deposition of secondary dentin, and a narrower apical foramen [1, 2, 3]. In contrast, immature permanent teeth exhibit unfinished root formation with an open apex, thinner dentin and enamel, a larger pulp chamber, and a wider apical foramen [2, 4, 5].

Primary dentinogenesis is the process by which the bulk of dentin is formed during tooth development, prior to eruption. Histologically, primary dentin is characterized by a regular, tubular structure with odontoblasts aligned in a palisaded layer at the pulp-dentin interface. The dentinal tubules are numerous, regularly spaced, and extend from the pulp toward the enamel or cementum [6]. Secondary dentinogenesis begins after root formation and tooth eruption, continuing throughout life at a slower rate. Secondary dentin is deposited along the entire pulpal surface, resulting in a gradual reduction of the pulp chamber. Histologically, secondary dentin is similar to primary dentin but shows a slight change in the direction of dentinal tubules at the junction, and a lower density of tubules compared to primary dentin. The thickening of secondary dentin is often most pronounced below the cemento-enamel junction, particularly in response to occlusal loading [7]. Tertiary dentinogenesis occurs in response to external stimuli such as caries, trauma, or restorative procedures [8]. Tertiary dentin can be classified as reactionary (formed by surviving odontoblasts) or reparative (formed by newly differentiated odontoblast-like cells after odontoblast death). Histologically, reactionary dentin may retain some tubular structure, though often irregular and less organized than primary or secondary dentin. Reparative dentin is typically atubular or contains sparse, irregular tubules, and may resemble fibrodentin or calcified scar tissue, especially after severe injury or pulp exposure [8]. These distinctions are critical for understanding the pulp-dentin complex's response to physiological aging and pathological insults.

Immature permanent teeth are more vulnerable to dental caries than fully developed permanent teeth due to their structural characteristics and increased porosity [9]. Their enamel is less mineralized, making it less resistant

to acid attacks. This condition allows carious lesions to advance rapidly into the dentin [10]. Furthermore, the post-eruptive enamel maturation process—which entails the absorption of mineral ions such as fluoride, calcium, and phosphate, and an increase in enamel hardness—remains incomplete in immature teeth. Consequently, this elevates the risk of caries [9].

Immature permanent teeth have larger pulp chambers and thinner dentin, which allows carious lesions to reach the dental pulp more quickly. This increases the risk of developing pulpitis or pulp necrosis [9, 10]. In contrast, fully formed teeth possess thicker, more mineralized enamel and dentin, along with a reduced pulp chamber volume. These features enhance resistance to the progression of caries and provide a longer buffer period before the dental pulp is affected [11, 12]. The American Academy of Pediatric Dentistry highlights the importance of the dynamic process of demineralization and remineralization, especially in newly erupted teeth, which are at a higher risk for the rapid and early development of dental caries [13].

The mesiobuccal pulp horn in newly erupted permanent teeth is of particular clinical importance due to its prominent extension toward the cusp tip, making it especially vulnerable to early carious involvement and iatrogenic exposure during cavity preparation. In newly erupted teeth, the overlying dentin is mainly primary dentin, which is relatively thin in the pulpal horn region, and the pulp chamber is large with prominent horns [14]. This anatomical configuration increases the risk of pulp exposure if caries progresses rapidly or if cavity preparation is not conservative [15]. The dentin thickness in the mesiobuccal pulp horn region significantly reduces the time available for the pulp to initiate a defensive response prior to exposure [15].

In cavity preparation, it is important to recognize the proximity of the mesiobuccal pulpal horn to the external surface and to preserve as much dentin as possible in this area. Excessive removal of carious or sound dentin can result in pulp exposure, especially before significant secondary or tertiary dentin has formed [16]. Selective caries removal and minimally invasive techniques are advised to reduce the risk of pulpal injury, as subclinical inflammation and bacterial penetration can occur even when a thin layer of dentin remains [8].

The structural integrity and functional stability of permanent teeth depend fundamentally on the thickness and distribution of their two primary hard dental tissues: enamel and dentin. Enamel, the hardest and most mineralized substance in the human body, serves as a protective outer layer. In contrast, dentin, a less mineralized but more resistant tissue, provides essential support and encases the vital pulp [17, 18]. Variations in the thickness of these tissues in specific anatomical regions—such as the tips of the cusps, above the pulp horns, and in the deepest part of the occlusal surface—are crucial for understanding the biomechanical characteristics of the tooth, its wear resistance, and its vulnerability to pathological conditions. These regions are particularly interesting because of their role in mastication, stress distribution, and susceptibility

to caries or fractures.

While the significance of enamel and dentin thickness is well-established, there remains a lack of studies that directly compare these structures in permanent teeth with complete versus incomplete root development. Additionally, there is a scarcity of studies that directly quantify secondary dentinogenesis. Immature teeth, which are still undergoing developmental processes such as mineralization, maturation, and root development, have different thickness profiles of their structures compared to their fully formed, mature counterparts. Understanding these differences is essential as it may influence clinical decision-making in the treatment of immature teeth in childhood and the development of age-specific dental materials.

## PURPOSE

The aim of the present study is:

1. To quantitatively assess and compare the volume of enamel, coronal dentin, and pulp chamber in mature and immature permanent molars.

2. To perform a comparative analysis of the thickness of enamel and dentin in the MB-cusp area and the central pit of the occlusal surface in immature and mature permanent molars.

## MATERIAL AND METHODS

The study included 48 extracted third permanent molars – 24 mature teeth in patients aged 25-30 and 24 immature teeth in patients aged 15-18. All molars were extracted for orthodontic purposes. Inclusion criteria for teeth in the study were third molars free of carious lesions, fractures, defects, and cracks. Informed consent was obtained from parents or patients. The study procedures received approval from the Ethics Committee of the Medical University of Sofia (Approval Number: No 1598/20. 05. 2022).

After extraction, the teeth were cleaned with hydrogen peroxide-soaked gauze and stored in 10% formalin until the start of the study. The roots of the specimens were then fixed using A-silicone impression material, and the crowns were scanned with a SkyScan Desktop X-ray Microtomograph (Bruker, Billerica, MA, USA) with an X-ray tube voltage of 100 kV, a beam current of 100  $\mu$ A, and a 0.55 mm copper filter. A conical beam with a voxel size of 12  $\mu$ m was used. At this resolution, the crowns were projected over their entire length into the detector field.

After scanning, a three-dimensional reconstruction of the enamel and dentin of the tooth crown was performed by superimposing a series of two-dimensional images and applying filters to correct the radiation spectrum and circular interference (NRecon software, Bruker). A noise reduction filter and segmentation were applied to separate the dental structures - enamel, dentin, and pulp. Using the software included with the device (CtAn, Bruker), morphometric measurements were performed, which included determining the volume of enamel and dentin in the coronal part, as well as the thicknesses of enamel and dentin in the area of †the MB-cusp and the deepest part of the fissure. The plane containing the most apical extension of the enamel was identified. In this study, the structures located

above that plane were defined as the crown. The crown volume data were recorded for each tooth, which was as follows: enamel volume, dentin volume, and pulp chamber volume. Then the volume ratio of the pulp chamber to the total crown was calculated.

**Statistical analysis:** The results obtained were subjected to statistical processing to compare the thicknesses of enamel and dentin and the volumes of dental structures of teeth with completed and incomplete root development. Data were analyzed using SPSS (v.26, IBM). Descriptive statistics (mean and standard deviation) were calculated for enamel and dentin volumes by tooth type. An independent samples T-test was conducted to compare these measurements across different tooth types. A p-value of less than 0.05 was considered to indicate statistical significance.

## RESULTS

Figure 1 shows the reconstructions of an immature and mature tooth.

**Fig. 1.** Reconstruction of enamel, dentin and pulp chamber in immature **A)** and mature **B)** molars. The less prominent and more pointed pulp horns in mature teeth are clearly visible.

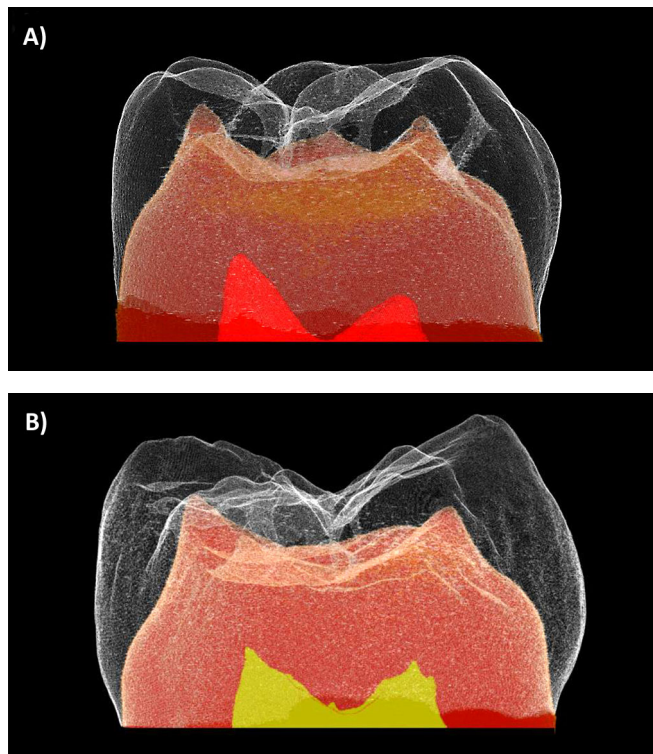


Table 1 shows the volumes of enamel and dentin of permanent molars with complete and incomplete root development.

**Table 1.** Volume of enamel and dentin in the studied groups (mm<sup>3</sup>)

| Teeth                 | Structure | Enamel           | Dentin           | Pulp           |
|-----------------------|-----------|------------------|------------------|----------------|
|                       |           | Mean ± SD        | Mean ± SD        | Mean ± SD      |
| <b>Immature teeth</b> |           | 244.529 ± 35.134 | 250.560 ± 47.025 | 19.759 ± 4.883 |
| <b>Mature teeth</b>   |           | 234.405 ± 49.457 | 309.884 ± 42.982 | 13.712 ± 3.443 |
| <b>T-test</b>         |           | p = 0.510        | p = 0.001        | p < 0.001      |

The results highlighted significant differences in dentin and pulp volume between the different groups of teeth, while enamel volume did not show significant variations. Enamel volume was slightly higher in immature teeth (244.529 ± 35.134 mm<sup>3</sup>) compared to mature teeth (234.405 ± 49.457 mm<sup>3</sup>), but the difference was not statistically significant (p = 0.510). In contrast, coronal dentin volume was significantly greater in teeth with complete root development (309.884 ± 42.982 mm<sup>3</sup>) compared to teeth

with incomplete root development (250.560 ± 47.025 mm<sup>3</sup>, p = 0.001), reflecting the accumulation of dentin with advancing age. The most pronounced difference was in the pulp volume, which was significantly smaller in mature teeth (13.712 ± 3.443 mm<sup>3</sup>) compared to the other studied group (p = 0.000).

Table 2 presents the variations in the thickness of enamel and dentin between the fissure area and the tip of the MB-cusp.

**Table 2.** Thickness of enamel and dentin in different areas in teeth with and without complete root development

| Teeth                 | Thickness | Tip of the MB-cusp |                | Deepest part of the fissure |               |
|-----------------------|-----------|--------------------|----------------|-----------------------------|---------------|
|                       |           | Enamel             | Dentin         | Enamel                      | Dentin        |
|                       |           | Mean ± SD          | Mean ± SD      | Mean ± SD                   | Mean ± SD     |
| <b>Immature teeth</b> |           | 2.060 ± 0.247      | 3.143 ± 0.4904 | 0.703 ± 0.237               | 2.697 ± 0.399 |
| <b>Mature teeth</b>   |           | 1.968 ± 0.396      | 3.500 ± 0.541  | 0.713 ± 0.297               | 3.259 ± 0.183 |
| <b>T-test</b>         |           | p = 0.220          | p = 0.003      | p = 0.867                   | p < 0.001     |

The table compares enamel and dentin thickness in permanent teeth with complete and incomplete development at two anatomical locations: the MB-cusp tip and the fissure's deepest part. The results show significant differences in dentin thickness, while enamel thickness remains without significant statistical differences. At the tip of the MB-cusp, the mean enamel thickness is slightly higher in immature teeth ( $2.060 \pm 0.247$  mm) compared to mature teeth ( $1.968 \pm 0.396$  mm), but the difference is not statistically significant ( $p = 0.220$ ). Similarly, in the deepest part of the fissure, enamel thickness does not show a significant difference ( $p = 0.867$ ). In contrast, such a difference is observed when comparing the dentin thickness. At the cusp tips, mature teeth had greater dentin thickness ( $3.500 \pm 0.541$  mm) than immature ( $3.143 \pm 0.4904$  mm,  $p = 0.003$ ). This difference was even more pronounced in the deepest part of the fissure ( $p < 0.001$ ).

## DISCUSSION

Using high-resolution imaging and precise measurement techniques, this study examined permanent teeth with complete and incomplete root development to quantify the thickness and volume of enamel and dentin, as well as to compare their structural parameters. By clarifying how the developmental stage influences tissue thickness in these critical areas, this study aimed to enhance our understanding of tooth maturation, secondary dentinogenesis, and their implications for dental health and treatment strategies.

The medical literature has not directly described the enamel volume of permanent teeth with complete and incomplete root development. However, enamel thickness and mineral content, which are the main determinants of enamel volume, have been quantitatively measured in some studies [19]. The average enamel thickness in permanent teeth is approximately 2.58 mm [19]. According to the data from our study, it is clear that differences in enamel thickness and volume in the two groups of teeth studied are not detected (Tables 1 and 2). Permanent immature teeth have enamel that is not fully mineralized and, therefore, less dense, but its final thickness is established before the tooth fully matures. The maturation process involves a significant increase in mineral density. However, the physical volume of enamel changes minimally after the secretory stage, and most of the changes are in the mineral content and porosity of enamel rather than in its volume [19, 20, 21]. Our data suggest that enamel volume remains relatively constant throughout tooth maturity (above the MB-pulpal horn and in the central fissure), consistent with its role as a stable, non-regenerating tissue formed before tooth eruption.

Factors such as diet and oral habits play a significant role in the rate of enamel abrasion [22]. In our study, we observed that there was minimal impact on enamel

thickness among the groups analyzed, likely due to the small age difference among the patients (Tables 1 and 2). Enamel wear generally progresses more noticeably over extended periods.

In contrast to enamel, significant differences were observed in dentin thickness between teeth with complete root development and those with incomplete root development, at both measurement sites: above the MB-pulpal horn, below the cusp, and in the deepest area of the fissure (Tables 1 and 2). These findings indicate that secondary dentin continues to be deposited over time, leading to increased dentin thickness as teeth age. This thickening is particularly evident in areas that are more susceptible to wear or decay, such as fissures and cusps. The mesiobuccal pulp horn is a high-risk site for pulp exposure in newly erupted permanent teeth due to its anatomy and the histological immaturity of the dentin-pulp complex, necessitating careful caries management and conservative cavity preparation.

Immature teeth, which have thinner dentin, are more susceptible to the progression of caries, particularly in fissures where the enamel is also thin (approximately 0.7 mm, Table 2). The reduced thickness of dentin in these incompletely developed teeth necessitates careful consideration of cavity preparation depth to avoid exposing the pulp, especially in uncooperative children. Additionally, significant differences were observed in the pulp chamber volume between the two groups of teeth. Specifically, the pulp volume was considerably larger in teeth with incomplete development (Table 1). This larger pulp volume, resulting from the absence of secondary dentin, heightens the risk of pulp exposure during restorative procedures, particularly in deep carious lesions located in fissures with thinner dentin (Table 2).

## CONCLUSION

This micro-CT study revealed no significant differences in enamel thickness or volume between permanent molars with complete and incomplete root development. In contrast, mature teeth demonstrated significantly thicker dentin layers above the pulp horns and in the central pit, alongside greater coronal dentin volume and a markedly reduced pulp chamber volume, driven by the ongoing process of secondary dentinogenesis. These structural changes highlight the protective role of secondary dentin deposition in mature teeth, which enhances resistance to caries and mechanical stress over time. Clinicians must exercise heightened caution during restorative procedures in teeth with incomplete root development to preserve the MB-pulpal horn, pulp-dentin complex and support long-term dental integrity.

## FUNDING

The article was developed with the support of a scientific project "Grant-2023", Contract No. 184/03. 08. 2023.

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*Please cite this article as:* Bogovska-Gigova R, Hristov K. Assessment of secondary dentinogenesis on permanent molars – a micro-CT study. *J of IMAB*. 2025 Jul-Sep;31(3):6361-6365. [Crossref - <https://doi.org/10.5272/jimab.2025313.6361>]

Received: 04/02/2025; Published online: 29/07/2025



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