*applied sciences*

MDPI

*Article*

# SEM/EDS Analysis of Tubules and Mineral Deposition in the Dentin of Children with Osteogenesis Imperfecta

**Andrea Martín-Vacas [1,2,*], Vicente Vera-González [3], Julio Ramírez-Castellanos [4], Diego González-Gil [5] and Manuel Joaquín de Nova García [6]**

[1] Faculty of Dentistry, Alfonso X El Sabio University, 28691 Villanueva de la Cañada, Spain
[2] Pediatric Dentistry Section, Department of Dental Clinical Specialties, Faculty of Dentistry, Complutense University of Madrid, 28040 Madrid, Spain
[3] Department of Conservative Dentistry and Prosthetics, Faculty of Dentistry, Complutense University of Madrid, 28040 Madrid, Spain
[4] Functional Inorganic Materials Group, Inorganic Chemistry Department, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain
[5] Surgery Department, Dental Clinic Faculty of Medicine, University of Salamanca, 37007 Salamanca, Spain
[6] Department of Dental Clinical Specialties, Faculty of Dentistry, Complutense University of Madrid, 28040 Madrid, Spain
* Correspondence: amartvac@uax.es or andrem02@ucm.es

**Abstract:** The aim is to quantitatively analyze the diameter and tubular density and semi-quantitatively analyze the elemental composition (Calcium and Phosphorus) in the dentin of primary teeth of children with Osteogenesis Imperfecta (OI) in comparison with a control group. Material and methods: A microstructural (in vitro) analysis of primary teeth of children with OI was performed with SEM and EDS. The variables measured were the tubule count (tubule/mm$^2$) and diameter (µm) at 2000 times magnification at four points of the dentin of different depths. A semiquantitative analysis of the elemental composition of the dentin was performed with EDS of Calcium and Phosphorus (cps). Descriptive and inferential analysis (Fisher's exact test, the Mann–Whitney U test, the Kruskal–Wallis test, the Bonferroni post hoc test, the ANOVA test of repeated measures, a test of inter- and intra-subject effects, and the Geisser–Greenhouse test) were carried out. Data were analyzed with a 95% confidence level ($p$-value < 0.05). Results: A total of 25 deciduous teeth from 17 patients with OI and 30 teeth from healthy children were studied. There were differences in the count and tubular diameter for the control group with OI; in addition, the behavior curve changed when the systemic disease was severe. While there were no differences in the amount of dentinal Calcium, the OI tooth showed a significantly lower amount of Phosphorus ($p < 0.05$), except in the pulpal dentin ($p > 0.05$). Conclusion: The alterations of the dentinal tubules (density and diameter) were more pronounced in the most severe phenotypes of systemic disease. The amount of Phosphorus was decreased in the dentin of the primary teeth of children with OI compared to the control group.

**Keywords:** osteogenesis imperfecta; dentistry; rare diseases; dentinogenesis imperfecta; primary teeth; pediatric dentistry

check for updates

**Citation:** Martín-Vacas, A.; Vera-González, V.; Ramírez-Castellanos, J.; González-Gil, D.; de Nova García, M.J. SEM/EDS Analysis of Tubules and Mineral Deposition in the Dentin of Children with Osteogenesis Imperfecta. *Appl. Sci.* **2023**, *13*, 12451. https://doi.org/10.3390/app132212451

Academic Editor: Andrea Scribante

Received: 7 October 2023
Revised: 12 November 2023
Accepted: 15 November 2023
Published: 17 November 2023



## 1. Introduction

Osteogenesis Imperfecta (OI) is a hereditary systemic disease, distinguished by a heterogeneous group of connective tissue disorders, which is characterized by the triad osteopenia, fragility, and bone deformity [1–3]. The low prevalence of OI (1:15,000–1:20,000 live births) without differences in sex, a popular affinity, makes it belong to the rare disease group [3–5]. Recently, new classifications based on the genetic mutation of patients have emerged, although the original classification by Sillence et al. [6] is still the most frequently used in clinical practice because it is simple and easy to use, favoring the intercommunication between professionals. According to this classification, there would be four OI types, depending on the clinical severity: mild non-deforming (type I), perinatal lethal (type II), severely deforming (type III) and moderately deforming (type IV) [6].

In the 1970s, Shields et al. [7] classified hereditary dentin defects into two large groups, with different subgroups: dentin-I dysplasia (DD) and Dentinogenesis Imperfecta (DGI). Of the three DGI groups, type I (DGI-I) is the structural alteration of dentin associated with OI [8,9], while type II occurs independently of systemic disease, and type III has been observed in an isolated population in southern Maryland and Washington D.C. The diagnosis of DGI-I is mainly clinical and radiographic (based on changes in dental color, bulbous crowns, marked cervical constriction, root changes and rapid pulpal obliteration, among other indicators). It is a very frequent finding in subjects with OI [10] and is more prevalent in the deciduous dentition than in the permanent one [11,12].

The etiopathological mechanism of OI is due to qualitative and/or quantitative alterations of type I collagen. The mutations occur in more than 90% of the cases in the genes that code for collagen alpha chains (COL1A1 and COL1A2) while in the remaining 10%, mutations occur in genes that interact with collagen during its metabolism [4,10,13–17]. Regarding the etiology of DGI-I, it is directly associated with OI and is one of the signs with the highest penetrance [9,18]. In previous studies, it has been hypothesized that the alteration of the dental microstructure is secondary to dysfunction of the odontoblasts due to intracellular accumulation of abnormal procollagen and collagen degradation products [11] or rapid obliteration of the dentinal tubules due to aberrant mineralization of the dentin [19]. In fact, the evidence seems to support the theory that the initial dentinal secretion would be normal if the formation of aberrant dentin begins later, since according to several authors [14,20,21], the outermost dentin has normal morphology, with a layer parallel to the dentin–enamel junction (DEJ) of dysplastic dentin continuing alternating dysplastic and tubular zones up to the pulpal tissue.

Although microstructural alterations are found in the enamel and DEJ in subjects with OI, DGI-I mainly manifests in dentin. In a study involving Spanish children with OI, it was determined that there were alterations in more than half of the deciduous teeth at the enamel (64% prevalence) and the DEJ level (64–72% prevalence), and in the dentin in all the analyzed teeth [20]. In addition, it has been confirmed that the microstructural alteration occurs even in teeth that do not appear to show signs of clinical and/or radiographic signs of DGI-I [19,20,22]. The dentin alterations described in the literature are mainly a decrease in tubular diameter and density, with an abnormal pattern in the direction of the dentinal tubules and a dysmorphic appearance [11,14,19,20]. The severity of dentin alteration has also been related to the systemic alteration (OI), with dentin being more dysmorphic in the most severe OI phenotypes [20].

The use of scanning electron microscopy (SEM) techniques for the analysis of the surface of the samples has multiple advantages (very good image quality and resolution, and ease of sample preparation, among others) but it requires training of laboratory personnel. There are seven studies that analyze the tissue morphology of deciduous teeth [11,14,19,20,22–25], but the numerical data of these variables and the comparison with a control group of healthy teeth is not conclusive. Regarding the variation in the mineral composition of DGI-I teeth, there is only one article [11] that determined the irregularities in the mineralization.

Even though dentin is the most studied dental tissue in patients with OI, previous research has biases mainly associated with the difficulty of sample collection due to the low incidence of OI. Currently, dental filling techniques with composite resins are based on micromechanical retention related to resin tags, so knowledge of the alteration of the distribution and disposition of the dentinal tubules is of vital importance for the elaboration of the treatment plan, although some authors [12,21] established that there is no contraindication in patients with DGI if enamel–composite adhesion is successful. In addition, the increase in knowledge of the dental microstructure of the DGI-I and its diffusion in the scientific health collective would allow better patient advice and more accurate interprofessional communication. Due to this, we proposed the study hypothesis that the teeth of subjects with OI (and therefore with manifest clinical and/or radiographic DGI) presented quantifiable tubular alterations and in dentin mineral deposition (specifically Calcium and Phosphorus). The aim is to carry out a quantitative analysis of the tubular diameter and density and a semiquantitative analysis of the elemental composition (Calcium and Phosphorus) in the dentin of deciduous teeth of children with OI in comparison with a control group.

## 2. Materials and Methods

The design of the in vitro study was carried out following the guidelines of the Declaration of Helsinki, for experimentation with humans, and approved by the Clinical Research Ethics Committee of the Hospital Clínico San Carlos (17/326-E Thesis Code). This research has been supported, though not economically, by the AHUCE Foundation (Association of Crystal Bones of Spain) in collaboration with the Complutense University of Madrid (UCM). The children and their parents/legal guardians were adequately informed by means of a written informed consent form.

### *2.1. Study Subjects*

The sampling carried out was non-probabilistic of consecutive cases, due to the low prevalence of OI, and was collected as part of the clinical activity of the Master of Pediatric Dentistry of the UCM between August 2017 and August 2018, inclusive. Additionally, due to the low prevalence of the systemic disease, and the heterogeneity of previous studies, no sample size calculation was performed. Deciduous teeth of children with a confirmed medical diagnosis of OI, physiologically exfoliated or extracted for justified reasons (caries, dental trauma, or orthodontic-eruptive reasons) were selected. The dental samples were immediately preserved after physiological exfoliation or dental extraction in a 35% reduced formaldehyde solution in a ratio of one quart with distilled water. Patients who, during the dental revision, expected dental exfoliation to occur in a short time were given a vial with the indicated solution for its preservation until it was sent or delivered directly to our dental service. The teeth extracted by the Master of Pediatric Dentistry were immediately preserved. Patients who refused to take part in the study or teeth that did not have enough remaining tissue to be studied were excluded. In accordance with establishing the normality of the dentin characteristics, control deciduous teeth from healthy children were collected, meeting the same selection criteria except for the systemic condition studied.

Patients were classified according to the type of OI according to the original classification of Sillence [6] in three types compatible with perinatal survival: OI type I (OI-I) or mild non-deforming, OI type III (OI-III) or severely deforming, and OI type IV (OI-IV) or moderately deforming. Data from the study subjects (medication, type of OI, dental alterations compatible with DGI-I) were recorded. The type of medication and genetic alteration were incorporated into the dental microstructural analysis. However, because the characteristics of dental alteration were assessed in the entire dentition of the children and not only in the collected teeth, this information was not analyzed. Furthermore, considering the evidence that all teeth from subjects with OI present clinical or subclinical DGI-I, it was not considered relevant.

### *2.2. Microstructural Analysis*

The dental samples were processed and analyzed at the National Center for Electron Microscopy in collaboration with the UCM. The entire methodological process of sample preparation comes from the internal protocol for analysis of biological mineralized structures, and according to dental tissues analysis with SEM [26]. The microstructural analysis was carried out using a JEOL-JSM 6400 SEM scanning microscope (JEOL Ltd., Tokyo, Japan), and was carried out within a maximum period of three months from the collection of each sample. Teeth were longitudinally sectioned in a vestibular–lingual direction [19,23] at the point of maximum dental contour to be able to analyze all points of the dentin of different depths. The longitudinal section allows us, in a single dental section, to visualize all the dentin from the pulp tissue to the dentin–enamel junction (DEJ). It was decided to carry out this section method due to dental fragility and the difficulty of obtaining teeth from subjects with OI. Furthermore, knowledge of the S-shape direction of the tubules would allow the view of the tubules in two different modes: longitudinal (cut parallel to the tubular direction) or transverse (cut perpendicular to the tubular direction). The surface was polished with silicon carbide abrasive discs (metalograph polishing machine 50–8435 MICRODUO-I AUTO, METALOGRAT, no data), in a decreasing sequence of the abrasion degree (disc 600, disc 800/2400 and disc 1200/4000). Subsequently, the teeth were treated with 37% orthophosphoric acid gel (Scotchbond$^{TM}$ etchant, 3M$^{TM}$ ESPE$^{TM}$, St. Paul, MN, USA) for 20s, evaporated (Quorum Q150T S$^{TM}$, Quorum Technologies, Lewes, UK) and plated with gold (Quorum Q150R S$^{TM}$, Quorum Technologies, Lewes, UK), to avoid surface charges. All the samples were collected, processed, and analyzed by the same operator (A.M.-V.), and previously calibrated.

The systematic analysis of the dentin was based on taking images at 2000 magnifications at four points of the dentin. In order to standardize the regions analyzed, the total thickness of the dentin was divided into four areas of equal thickness, and the analysis was conducted in four portions, from the most external to the most internal. These were referred to as external or outer, middle, deep and pulpal dentin (adjacent to pulpal tissue), respectively. Because a longitudinal section of the tooth is not the best way to visualize the tubules—although, due to its serpentine shape, it allows, in many cases, a transverse view of them—an approximation to the tubular density and diameter was made. At each point of the dentin, the dentinal tubule count was recorded (initially in $\mu m^2$ and converted to $mm^2$), and the diameters (µm) of the tubules present in the image were measured with the ImageJ/Fiji software for Windows (https://fiji.sc/, No copyright, accessed on 8 March 2017), obtaining the average in each microscopic section. It was anticipated that due to the meandering path of the dentinal tubules, some cuts would obtain a cross section and others a longitudinal section, affecting the variables of count and tubular diameter, so the cutting pattern was included as a variable, eliminating its action as analysis bias.

### 2.3. Mineral Analysis

A semiquantitative analysis of the elemental composition was carried out using energy dispersion spectroscopy (EDS) with the SEM, with a resolution of 133 eV, obtaining the results in counts per second (cps) of the elements Calcium and Phosphorus in the four mentioned points of the dentin.

### 2.4. Statistical Analysis

Descriptive statistics of the data obtained through counts, percentages, means, and standard deviations (SD) were performed, depending on the nature (qualitative or quantitative) of the variable. The intra-operator concordance was evaluated in 25 random samples (45.45% of the sample) with the intraclass correlation coefficient (ICC). Qualitative data were analyzed with Fisher's exact test. Quantitative data were subjected to normality tests (Shapiro–Wilk test with Lilliefors correction) and, later, to the Mann–Whitney U test and Kruskal–Wallis test, for independent samples, and the Bonferroni post hoc test was used for the comparisons between study groups. The effect of the type of tooth and the depth of the dentin was analyzed with the ANOVA test of repeated measures (with Bonferroni multiple comparisons adjustment), including analysis with the test of inter-subject effects, test of intra-subject effects and the Geisser–Greenhouse (G-G) test for paired comparisons. Data were analyzed with the SPSS® version 25 for Windows program (IBM Corp. Released 2017. IBM® SPSS® Statistics for Windows, Version 25.0. IBM Corp., Armonk, NY, USA) with a 95% confidence level ($p$ value $< 0.05$) and asymptotic or bilateral significance (except Fisher's test, with exact significance).

## 3. Results

### 3.1. Sample Characteristics

A total sample of 25 deciduous teeth from children with OI and 30 teeth from healthy subjects (control sample) was obtained; the distribution and average age at which the samples were obtained can be seen in Table 1. The distribution of the samples in terms of sex ($p$ Fisher = 0.265) and tooth type ($p$ Fisher = 0.745) was homogeneous in the two study groups. The distribution of the type of tooth was also homogeneous, considering the type of OI ($p$ Fisher = 0.631). Of the teeth collected, 96% came from subjects who were undergoing medical pharmacological treatment with bisphosphonates, and 77.8% of the sample had a genetic diagnosis with COL1A1-COL1A2 alteration.

**Table 1.** Demographic description of the study sample.

| | | Tooth | | | Obtaining Age | | Bisphosphonate Treatment | | Genetic Mutation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Incisor | Canine | Molar | Mean | SD * | Yes | No | CO1A1-COL1A2 | Other | Unknown |
| Study (OI) sample | OI-I | 4 | 3 | 4 | 9.22 | 3.34 | 10 | 1 | 7 | 2 | 2 |
| | OI-III | 2 | 4 | 4 | 11.20 | 3.77 | 10 | - | 5 | 2 | 3 |
| | OI-IV | 3 | 1 | - | 7.44 | 1.54 | 4 | - | 2 | - | 2 |
| | Total | 9 | 8 | 8 | 9.73 | 3.49 | 24 | 1 | 14 | 4 | 7 |
| Control sample | | 8 | 10 | 12 | 10.14 | 2.64 | - | 30 | - | - | - |

* SD—standard deviation.

Although the study was based on dental samples, the population was also analyzed. The study sample (OI) came from 17 children with OI and the control sample from 30 healthy children. In compliance with the inclusion criteria, none of the children in the control sample were taking systemic medication or had dental alterations.

Children with OI (eight boys and nine girls) were medicated with bisphosphonates in 94.1% of cases, and 58.8% had a genetic diagnosis with an alteration of the COL1A1-COL1A2 gene. Clinical dental alterations (alteration of color, severe attrition or appearance of bulbous crowns) were found in 41.2% of the children studied, and radiographic alterations (alteration of root formation, pulp obliteration, dental agenesis) in 69.2%.

### 3.2. Microstructural Analysis

An intra-operator reliability analysis of the study variables was performed, obtaining an ICC = 0.996 for the tubular count variable, and an ICC = 0.988 for the tubular diameter variable, considering both values as an excellent degree of agreement [26]. We aimed to determine whether the distribution of the variables tubular count and diameter followed normal criteria, and found that although most of the variables followed a normal curve, the tubular count did not have a normal distribution in nine of the points studied, and the tubular diameter was observed at four of the points studied ($p$ Shapiro–Wilk < 0.05). In the analysis of the detection of Calcium and Phosphorus, normality criteria were not met in most of the study points ($p$ Shapiro–Wilk < 0.05).

It was found that the type of tooth influenced the count or tubular density in the longitudinal cut pattern in the external ($p$ value = 0.046), deep ($p$ value = 0.032) and pulpal ($p$ value = 0.028) dentin. The differences found in the average dentin or in the cross sections and in the variable tubular density were not statistically significant, so it was secondary to the type of cut of the dentinal tubules, and not an effect of the type of tooth. Due to this, the type of tooth was not differentiated in the analysis of the variables.

The differences in the tubular count are not statistically significant in the longitudinal cut pattern or in the OI-control group comparison, neither between the three OI groups, excluding or not the control group ($p$ value > 0.05) (Table 2). In the cross-sectional pattern, there are significant differences between control and OI groups in the external ($p$ U Mann–Whitney = 0.003) and pulpal dentin ($p$ U Mann–Whitney = 0.027), with a higher tubular density of the teeth with OI in external dentin and lower in pulpal dentin than healthy teeth (Table 2). Regarding OI types, statistically significant differences were found in the tubular count in outer or external dentin between healthy controls and OI-I, and in deep and middle dentin between OI-I and OI-III ($p$ value < 0.05). The results suggest the existence of a higher overall tubular density and in the deep dentin in the subjects with OI-I compared to OI-III, in addition to a higher tubular count in the outermost dentin than the control group.

**Table 2.** Tubular count of the control and study sample, measured in tubules/mm$^2$.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **LONGITUDINAL PATTERN** | | | | | | | | | | |
| | **External Dentin** | | **Middle Dentin** | | **Deep Dentin** | | **Pulpal Dentin** | | **Dentin (Average)** | |
| | **Mean** | **SD [#]** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **Control sample ($n$ = 18)** | | | | | | | | | | |
| **Total** | 718.84 | 819,99 | 4660.86 | 2105.29 | 5565.21 | 2429.57 | 6052.16 | 2784.34 | 4813.52 | 1665.51 |
| **Study sample (n = 8)** | | | | | | | | | | |
| **OI-I** | 1669.56 | 2286.14 | 5113.03 | 2370.32 | 8973.89 | 4236.05 | 8347.81 | 3356.47 | 6426.07 | 2648.56 |
| **OI-III** | 1669.56 | 2182.17 | 4486.95 | 1500.09 | 4799.99 | 3878.21 | 6886.94 | 5218.77 | 5008.68 | 2671.40 |
| **OI-IV** | - | - | - | - | - | - | - | - | - | - |
| **Total** | 1669.56 | 2068.99 | 4799.99 | 1866.63 | 6886.94 | 4371.94 | 7617.37 | 4136.47 | 5717.38 | 2576.59 |
| **Inter- and intra-group statistical significance** | | | | | | | | | | |
| **Control-OI [a]** | 0.461 | | 1 | | 0.495 | | 0.311 | | 0.641 | |
| **Control-OI Type [b]** | 0.730 | | 0.989 | | 0.222 | | 0.406 | | 0.419 | |
| **OI Types [b]** | 0.886 | | 1 | | 0.343 | | 0.686 | | 0.486 | |
| **CROSS PATTERN** | | | | | | | | | | |
| | **External dentin** | | **Middle dentin** | | **Deep dentin** | | **Pulpal dentin** | | **Dentin (average)** | |
| | **Mean** | **SD [#]** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **Control sample ($n$ = 12)** | | | | | | | | | | |
| **Total** | 313.04 | 619.73 | 7060.85 | 3130.85 | 11,930.4 | 7441.98 | 15,165.18 | 7473.57 | 10,498.53 | 4554.90 |
| **Study sample ($n$ = 17)** | | | | | | | | | | |
| **OI-I** | 4650.92 | 3453.92 | 10,255.8 | 5284.33 | 16,755.2 | 7130.04 | 12,939.1 | 13,655.32 | 12,596.25 | 5144.49 |
| **OI-III** | 4034.77 | 4907.96 | 3826.08 | 4067.50 | 4660.86 | 6226.91 | 4660.86 | 8370.39 | 4869.55 | 4574.19 |
| **OI-IV** | 2399.99 | 2345.69 | 4591.29 | 4997.08 | 6886.94 | 3582.43 | 6991.29 | 4066.43 | 5608.68 | 2375.48 |
| **Total** | 3903.83 | 3721.40 | 6653.69 | 5482.91 | 10,164.68 | 8163.08 | 8617.88 | 10,470.12 | 8225.05 | 5640.39 |
| **Inter- and intra-group statistical significance** | | | | | | | | | | |
| **Control-OI [a]** | 0.003 * | | 0.777 | | 0.471 | | 0.027 * | | 0.211 | |
| **Control-OI Type [b]** | 0.020 * | | 0.142 | | 0.013 * | | 0.076 | | 0.013 * | |
| **OI types [b]** | 0.594 | | 0.121 | | 0.013 | | 0.269 | | 0.017 * | |

[#] SD. Standard Deviation.; [a] Exact and asymptotic significance of the Mann–Whitney U Test; [b] Asymptotic significance of the Kruskal–Wallis Test; * $p < 0.05$. Statistical significance.

The behavior of the tubular density in the dentin points studied was analyzed, and a similar curve was observed in the longitudinal cut pattern in the control group and the group with OI ($p$ G-G = 0.611) and in the three OI types ($p$ G-G= 0.36) (Figure 1) with an increase in the tubular count as the dentin approaches the pulpal tissue. Significant differences ($p$ G-G < 0.001) were found between the outer dentin and the rest of the studied points, and between the medium and deep dentin. In the cross-sectional pattern, on the other hand, the curve shows a different behavior between the two groups studied ($p$ G-G = 0.015) and the types of OI ($p$ G-G = 0.034), since while in the control group the tubular density increases from the exterior to the interior of the tooth with significant differences between the external dentin and the rest of the study points ($p$ G-G <0.001), in the OI group there is an observable decrease in the tubular count in the pulpal dentin (Figure 1).

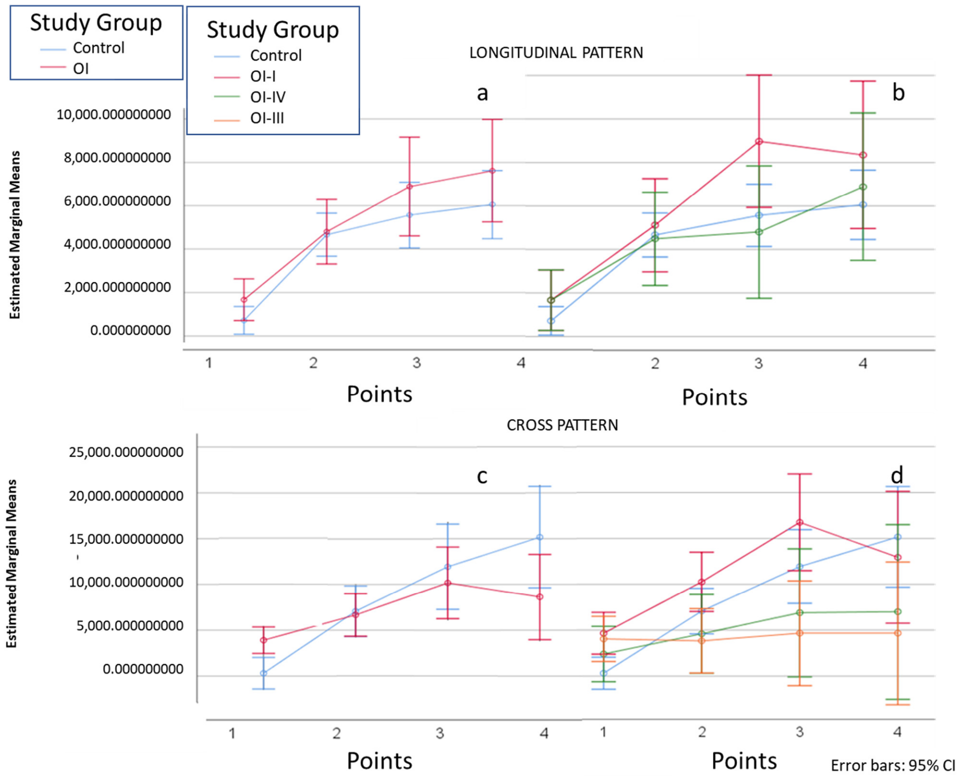


**Figure 1.** Behavior curve of the tubular count in the four studied dentin points in the longitudinal (**a**,**b**) and cross (**c**,**d**) tubular cut pattern with respect to the control group (**a**,**c**), and to the OI types and the control group (**b**,**d**).

Regarding the tubular diameter, there are no statistically significant differences between the teeth of control subjects and those with OI, between the OI types and the controls, nor between the OI types between them in the longitudinal cut pattern ($p$ value > 0.05). In the cross-sectional pattern, significant differences were found, since the diameter of the tubules in the external dentin is smaller in the control group (0.59 µm) than in the teeth of subjects with OI (1.58 µm) ($p$ U Mann–Whitney = 0.018) and OI-I (2.07 µm) ($p$ Bonferroni = 0.022) (Table 3). In the deep dentin, a greater tubular diameter is found in the teeth of OI-III (2.14 µm) compared to OI-I (3.49 µm) ($p$ Bonferroni = 0.049).

The behavior curve of the tubular diameter in the dentin was similar in the control and OI group ($p$ G-G = 0.638), and between the control and the OI types ($p$ G-G 0.787), and an initial significant increase was found in the tubular diameter from the outer to the middle dentin which then decreased in the pulpal tissue ($p$ G-G < 0.001). However, in the cross-sectional pattern, the diameter curve does not follow a similar behavior between the two study groups ($p$ G-G = 0.002) or between the OI types ($p$ G-G = 0.001) (Figure 2). Both in the OI group and control, an increase in tubular diameter is observed from the outer dentin to the middle, which then gradually decreases to the pulpal tissue ($p$ G-G < 0.05); however, in the control group, there are significant differences between the outer dentin and the other points studied, while in the OI group, the differences were not significant between the outer and pulpal dentin. Analyzing the OI type, the OI-I and OI-IV groups presented a significantly smaller tubular diameter in the external dentin with respect to the deep one ($p$ G-G = 0.005 and $p$ G-G = 0.012, respectively).

**Table 3.** Tubular diameter of the control and study sample, measured in μm.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **LONGITUDINAL PATTERN** | | | | | | | | | | |
| | **External Dentin** | | **Middle Dentin** | | **Deep Dentin** | | **Pulpal Dentin** | | **Dentin (Average)** | |
| | **Mean** | **SD [#]** | **Mean** | **SD** | **Mean** | **Mean** | **SD** | **Mean** | **SD** | **Mean** |
| **Control sample ($n$ = 18)** | | | | | | | | | | |
| **Total** | 1.64 | 1.43 | 3.70 | 0.56 | 3.23 | 0.74 | 2.81 | 0.79 | 2.82 | 0.64 |
| **Study sample ($n$ = 8)** | | | | | | | | | | |
| **OI-I** | 1.87 | 1.81 | 3.63 | 0.95 | 2.97 | 1.04 | 2.46 | 0.85 | 2.73 | 0.95 |
| **OI-III** | 1.24 | 1.44 | 3.84 | 0.64 | 2.77 | 1.08 | 2.37 | 0.39 | 2.55 | 0.57 |
| **OI-IV** | - | - | - | - | - | - | - | - | - | - |
| **Total** | 1.55 | 1.55 | 3.73 | 0.76 | 2.87 | 0.99 | 2.42 | 0.61 | 2.64 | 0.73 |
| **Inter- and intra-group statistical significance** | | | | | | | | | | |
| **Control-OI [a]** | 0.683 | | 0.807 | | 0.605 | | 0.238 | | 0.765 | |
| **Control-OI type [b]** | 0.840 | | 0.937 | | 0.770 | | 0.406 | | 0.669 | |
| **OI types [b]** | 0.886 | | 1 | | 0.886 | | 0.686 | | 0.486 | |
| **CROSS PATTERN** | | | | | | | | | | |
| | **External dentin** | | **Middle dentin** | | **Deep dentin** | | **Pulpal dentin** | | **Dentin (average)** | |
| | **Mean** | **SD[#]** | **Mean** | **SD** | **Mean** | **Mean** | **SD** | **Mean** | **SD** | **Mean** |
| **Control sample ($n$ = 12)** | | | | | | | | | | |
| **Total** | 0.59 | 0.87 | 3.48 | 0.58 | 3.08 | 0.65 | 2.62 | 0.61 | 2.44 | 0.38 |
| **Study sample ($n$ = 17)** | | | | | | | | | | |
| **OI-I** | 2.07 | 1.04 | 3.48 | 0.52 | 3.49 | 0.56 | 1.85 | 1.38 | 2.72 | 0.65 |
| **OI-III** | 1.25 | 1.25 | 2.08 | 2.17 | 2.04 | 1.18 | 2.04 | 0.8 | 1.85 | 0.95 |
| **OI-IV** | 1.21 | 0.94 | 1.86 | 1.49 | 3.44 | 1.3 | 2.72 | 0.56 | 2.31 | 1 |
| **Total** | 1.58 | 1.12 | 2.61 | 1.6 | 2.97 | 1.17 | 2.12 | 1.05 | 2.32 | 0.88 |
| **Inter- and intra-group statistical significance** | | | | | | | | | | |
| **Control-OI [a]** | 0.018 * | | 0.227 | | 0.983 | | 0.263 | | 0.913 | |
| **Control-OI type [b]** | 0.037 * | | 0.156 | | 0.062 | | 0.333 | | 0.341 | |
| **OI types [b]** | 0.269 | | 0.241 | | 0.041 * | | 0.392 | | 0.345 | |

[#] SD—standard deviation.; [a] Exact and asymptotic significance of the Mann–Whitney U Test; [b] asymptotic significance of the Kruskal–Wallis Test; * $p < 0.05$. Statistical significance.

### 3.3. Mineral Analysis

The EDS analysis showed that there were no significant differences in the amount of dentinal Calcium between the control group and the OI group ($p$ U Mann–Whitney > 0.05), nor between the OI types ($p$ Kruskal–Wallis > 0.05). Regarding dentin Phosphorus, a significantly lower amount of Phosphorus was found in the OI group than in the control group ($p$ U Mann–Whitney < 0.05) in all the locations studied except in the pulpal dentin. When analyzing the OI types, differences were found in the outer or external dentin ($p$ Kruskal–Wallis = 0.018) between the group with OI-IV and the controls ($p$ Bonferroni < 0.05) (Table 4).

The distribution of Calcium in the four study points was similar in the control and OI group ($p$ G-G = 0.257), and in the OI types ($p$ G-G = 0.352), increasing from the outermost to the deepest dentin, and then decreasing in pulpal dentin (Figures 3 and 4). The behavior of the Phosphorus in the four points of the dentin followed a similar curve in the control group and OI ($p$ G-G = 0.111), and in the OI types ($p$ G-G = 0.36). A lower amount of Phosphorus was observed in the pulpal dentin compared to the middle and deep dentin in the general group ($p$ G-G < 0.001), and between the deep and pulpal dentin in the OI types ($p$ G-G = 0.005). It was found that at all points, the amount of Phosphorus was lower in the OI group ($p$ intersubject effects = 0.025).

**Table 4.** Detection of calcium and phosphorus in the control and study sample. Measured in cps.

| | | External Dentin | | Middle Dentin | | Deep Dentin | | Pulpal Dentin | | Dentin (Average) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD [#] | Mean | SD | Mean | Mean | SD | Mean | SD | Mean |
| CALCIUM DETECTION | Control sample | | | | | | | | | | |
| | Total | 4.52 | 3.93 | 5.59 | 5.10 | 6.03 | 5.70 | 2.74 | 2.89 | 4.72 | 3.88 |
| | Study sample | | | | | | | | | | |
| | OI-I | 3.13 | 1.30 | 4.72 | 3.43 | 4.17 | 2.65 | 1.79 | 1.65 | 3.45 | 1.58 |
| | OI-III | 3.15 | 2.31 | 4.27 | 4.18 | 4.85 | 6.72 | 3.98 | 4.53 | 4.06 | 4.17 |
| | OI-IV | 1.53 | 0.62 | 4.56 | 6.48 | 6.12 | 5.67 | 3.87 | 3.8 | 4.02 | 3.87 |
| | Total | 2.88 | 1.76 | 4.52 | 4.09 | 4.76 | 4.94 | 3.00 | 3.44 | 3.79 | 3.08 |
| | Inter- and intra-group statistical significance | | | | | | | | | | |
| | Control-OI [a] | 0.302 | | 0.254 | | 0.335 | | 0.761 | | 0.478 | |
| | Control-OI type [b] | 0.357 | | 0.379 | | 0.629 | | 0.485 | | 0.856 | |
| | OI types [b] | 0.246 | | 0.316 | | 0.629 | | 0.196 | | 0.851 | |
| PHOSPHORUS DETECTION | Control sample | | | | | | | | | | |
| | Total | 6.01 | 4.44 | 6.85 | 5.36 | 7.21 | 6.32 | 4.36 | 3.76 | 6.11 | 4.65 |
| | Study sample | | | | | | | | | | |
| | OI-I | 3.38 | 0.93 | 4.82 | 2.98 | 4.39 | 2.61 | 2.96 | 2.87 | 3.89 | 1.61 |
| | OI-III | 3.10 | 2.30 | 3.68 | 3.23 | 4.04 | 4.86 | 3.48 | 3.39 | 3.57 | 3.32 |
| | OI-IV | 1.72 | 0.69 | 3.93 | 5.29 | 5.00 | 4.48 | 3.28 | 3.01 | 3.48 | 3.26 |
| | Total | 3.00 | 1.66 | 4.22 | 3.38 | 4.35 | 3.78 | 3.22 | 2.99 | 3.70 | 2.56 |
| | Inter- and intra-group statistical significance | | | | | | | | | | |
| | Control-OI [a] | 0.006 * | | 0.012 * | | 0.029 * | | 0.079 | | 0.016 * | |
| | Control-OI type [b] | 0.018 * | | 0.045 * | | 0.112 | | 0.342 | | 0.084 | |
| | OI types [b] | 0.158 | | 0.224 | | 0.463 | | 0.929 | | 0.483 | |

[#] SD—standard deviation. [a] Exact and asymptotic significance of the Mann–Whitney U Test; [b] asymptotic significance of the Kruskal–Wallis Test; * $p < 0.05$—statistical significance.

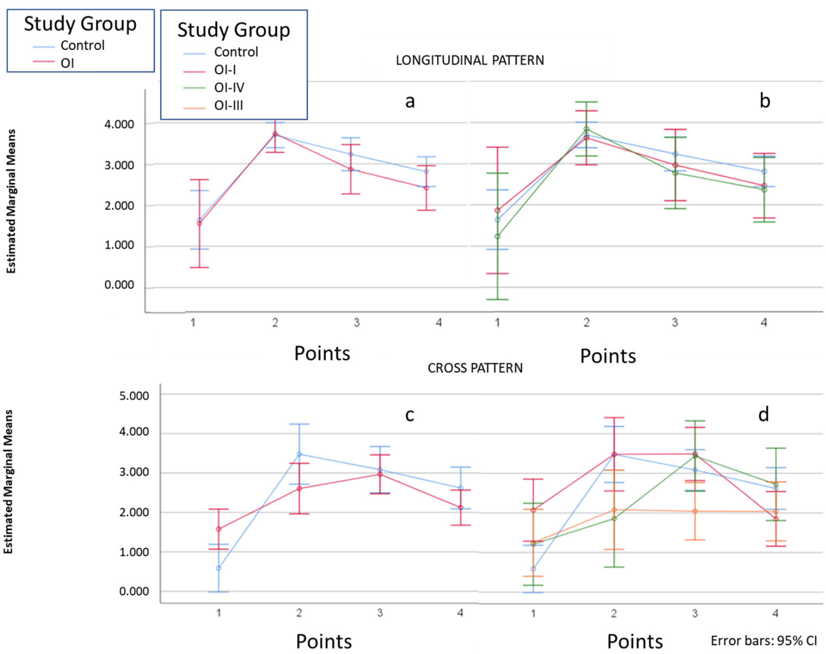


**Figure 2.** Behavior curve of the tubular diameter in the four points of the dentin studied in the longitudinal (**a**,**b**) and cross (**c**,**d**) tubular cut pattern with respect to the control group (**a**,**c**) and with respect to the types of OI and the control group (**b**,**d**).

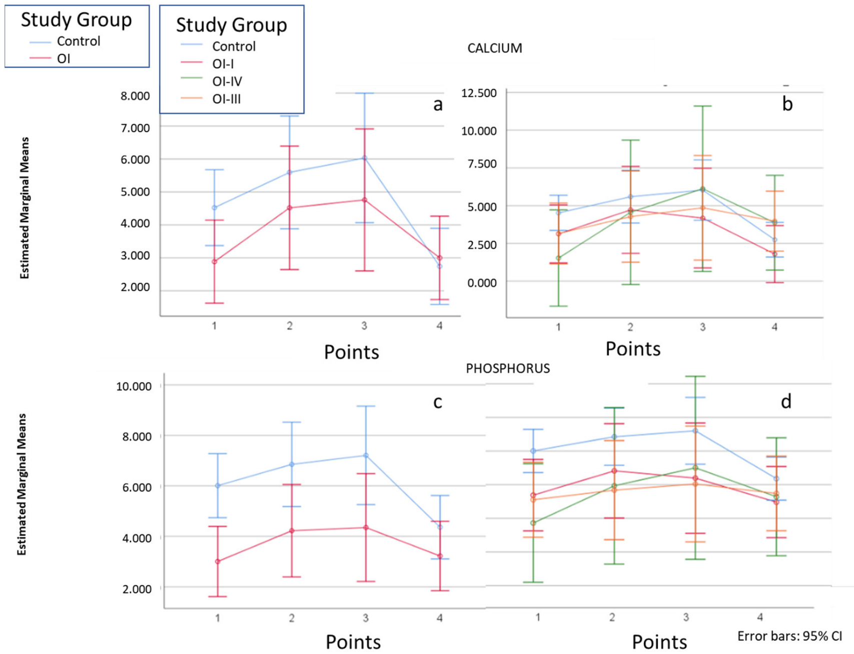


**Figure 3.** Behavior curve of Calcium (**a**,**b**) and Phosphorus (**c**,**d**) in the four studied dentin points with respect to the control group (**a, c**) and with respect to the OI types and the control group (**b**,**d**).

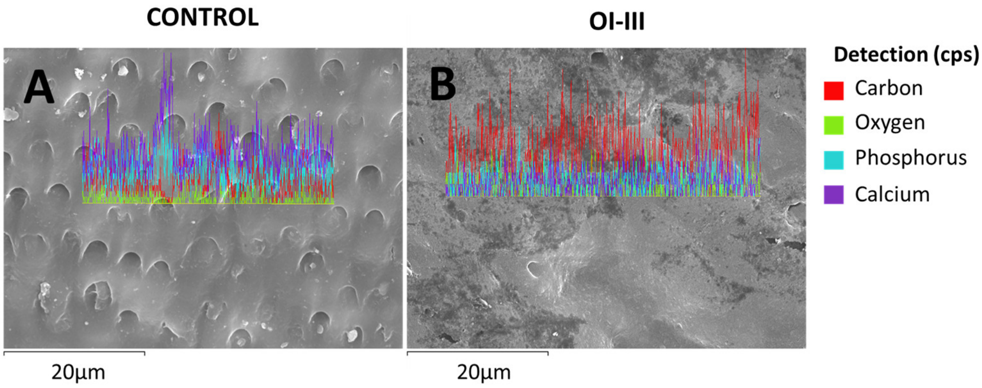


**Figure 4.** SEM images from the analysis conducted via energy dispersion of X-rays (EDS) of deep dentin at 2000× magnification of a primary second molar from the control sample (**A**) and from a patient with OI-III (**B**). The difference in the proportion of mineral components and in tubular characteristics (diameter and density) can be observed.

## 4. Discussion

The dental morphological and microstructural characteristics of the deciduous teeth of patients with OI have great variability; it is also present in patients who clinically or radiographically do not show DGI-I. Dental microstructural characteristics in dental hard tissues were studied and described by our study group [20]. Because in our previous studies it was observed that all the primary teeth of subjects with OI have ultrastructural alterations, the clinical dental and/or radiographic characteristics were not collected, since these are subjective to analyze and evolve (worsen) with the age of the patients (e.g., attrition, pulp obliteration, etc.). However, it seemed relevant to study the characteristics of the dentinal tubules (density and diameter) and the mineral quantity in terms of Calcium and Phosphorus of the dentin in comparison with a control group. To achieve this, SEM images at 2000 magnification with excellent resolution were used. In addition, the analysis via energy dispersion of X-rays (EDS) allows us to study the chemical composition of the particles and the distribution of the chemical elements on the surface of the sample. The high ICC results obtained establish that the method used is reliable and reproducible.

Analyzing the results, it is possible to highlight the size obtained in the sample, which was made up of 25 deciduous teeth of children with OI and 30 in the control group. In previous studies, the sample size ranged from 1–26 deciduous teeth [11,14,19,23,27–30], with a control group only in three of them [19,27,28]. Although most studies used SEM for ultrastructural analysis, there are others that used confocal laser scanning microscopy (CLSM) [11] or transmission electron microscopy (TEM) [27,28].

Regarding the diameter and tubular density in healthy patients, it is concluded that the dentinal tubules have a greater diameter and density in the region closest to the pulp, decreasing progressively to the region closest to the DEJ [31–34]. Studies on the dentin microstructure in primary dentition present heterogeneous data, since the regions studied, the methodology and the study sample vary between previous investigations; for this reason, with the aim of discussing the results objectively, only research performed with SEM and primary teeth will be discussed.

Analyzing the microanatomical dentin characteristics of healthy patients, we found that the average tubular density in healthy teeth in our study was lower than described by other previous authors [35–38], with a mean density of 4813.52 $\pm$ 1665.51 tubules/mm$^2$ in longitudinal sections and 10,498.53 $\pm$ 4554.90 tubules/mm$^2$ in cross sections. Regarding the tubular diameter, our data indicated a mean diameter of 2.82 $\pm$ 0.64 µm and 2.44 $\pm$ 0.38 µm in the longitudinal and transversal cut patterns, respectively, like those obtained in the study of Schilke et al. [36], but they differ with other studies that report slightly higher [38] or lower [35,37] tubular diameters than our results. Regarding the depth of analysis in the dentin, our results reveal that an increase in tubular density occurs when approaching the pulpal tissue, coinciding with Koutsi et al. [35], Sumikawa et al. [31] and Shilke et al. [36], but not with Lenzi and his research group [38], who did not find significant differences in terms of tubular density and dentin analysis depth. In relation to the tubular diameter in healthy patients, our results indicate that the largest pulpal diameter is found in the middle dentin, gradually decreasing up to the dentinal–enamel junction (DEJ), on the one hand, and when approaching the pulpal tissue, on the other hand. Our results contradict previous studies [31,35,36,38], since it is described that the largest tubular diameter is found in the region of dentin closest to the pulp and gradually decreases when moving away from it. The variations related to the density and tubular diameter found with other authors may be due to the non-standardization of the places used for the measurement, the non-coincidence of the sample preparation methodology, or the extrapolation of the results measured at different magnifications with the SEM.

The data obtained seem to indicate that in cross sections of teeth from subjects with OI, there is a higher mean count of dentinal tubules in the outermost dentin, and a lower mean count in the innermost dentin (pulp) than in the teeth of healthy subjects. Analyzing the OI type, a lower mean tubular count was found in the more severe phenotype of the systemic disease (OI-III) than in the milder one (OI-I). These differences are also significant with respect to the controls for the OI-I group in the outermost dentin, and for the OI-III group in the deep dentin. Even though various authors describe a decrease in tubular density in patients with OI [14,19,23,29,30], none of the studies treat this variable in a numerical analytical way.

In this research, no significant differences were found between the diameter of the controls and the teeth of OI subjects in total dentin; however, tubules with a significantly larger diameter are found in the external dentin of teeth of OI patients (1.58 µm) compared to healthy teeth (0.59 µm). Analyzing the OI types, a significantly higher tubular diameter was found in the external and deep dentin of the OI-I group compared to the control and OI-III group, respectively. Regarding the previous literature regarding tubular diameter, most authors agree that it is decreased in patients with OI [23,29] even less than 1 µm [19]; however, there is no consensus with the presence of other structures. Lygidakis et al. [19] also found elongated tubules with a tortuous and irregular trajectory, and other authors described the presence of structures like canals with a wide diameter (5–25 μm) [14], also detected with TEM [28] and with confocal laser scanning microscopy (CLSM) [11].

In the cross-sectional pattern, significant differences were detected in the behavior of both the diameter and the tubular density between the control sample and the OI teeth. In both cases, the difference seems to respond to an irregular pattern, far from normal, in which a model of severity associated with the severity of the systemic disease cannot be established Lygidakis et al. [19] hypothesizes that the reduction in tubular density may be because the dentinal tubules do not completely penetrate the thickness of the dentin, causing an excess of intertubular dentin. Indeed, the heterogeneity observed in the pattern of tubular diameter and density at different levels of dentin depth seems to be associated with abnormalities in intertubular dentin secretion and the direction and penetration of abnormal dentinal tubules.

A semi-quantitative elemental analysis (in the absence of standard standards) was carried out using EDS of the amount of Calcium and Phosphorus in control teeth, measured in counts per second (cps). It was found that there was an average of $4.72 \pm 3.88$ cps of Calcium and $6.11 \pm 4.65$ cps of Phosphorus: the amount of Phosphorus exceeding that of Calcium, contradicting previous studies in both temporary and permanent dentition [39–41]. These data indicate that the amount of calcium and phosphorus increases from the UAD to the deep dentin region, followed by a decrease in the dentin closest to the pulp, coinciding with being the last dentin secreted and, therefore, the lower mineralization of the same can be explained, since the mechanisms of secretion and mineralization of circumpulpal dentin are continuous and constant throughout the life of the tooth.

In this study, the amount of Calcium and Phosphorus was analyzed, and it was determined that there are no differences in the amount of Calcium between teeth with OI and healthy ones. However, it was found that in all regions except pulpal dentin, the amount of Phosphorus was significantly lower than that found in the same locations of healthy teeth; we also found a mean amount of Phosphorus that was significantly lower than the control group. Regarding the type of OI, it has been established that there is a lower amount of Phosphorus in the teeth with OI-IV than in the control teeth in the outer dentin, with no significant differences in the other study points or with the other OI types. The behavior of the amount of Calcium and Phosphorus in the four study points was studied, and it was determined that there was an increase in Calcium and Phosphorus from the external dentin to the deep one, which would later descend again, exhibiting the same behavior as the control group in both cases. Only the research of Majorana et al. [11] was found in relation to studying a primary tooth with CLSM; in their work, they stated that the dentin of OI subjects presents less mineralization, in accordance with our results, and they explain this fact by establishing that the increase in type III collagen could be an obstacle to mineralization. On the other hand, a recent study carried out with Raman spectroscopy in deciduous teeth with mild forms of OI without clinical signs of DGI-I showed an increase in the hardness of OI dentin associated with an increase in mineral content compared to healthy patients [42].

This research has important strengths, such as the presentation of a complete microanalysis of the dentin of deciduous teeth from subjects with a rare disease, OI. The results that demonstrate the differences in the pattern and dentinal mineralization of these teeth allow us to improve our knowledge of the dental affectation of the OI, facilitating a better dental therapeutic approach. The arrangement of 25 deciduous teeth of subjects with OI with a control group is exceptional; this was made possible thanks to the collaborative agreement between the Complutense University of Madrid and AHUCE, and the involvement of the families of children with OI.

This research also has limitations, such as the small size of the study sample associated with the rare disease condition of OI. In addition, in recent decades, it has been proposed to move away from the clinical classification of systemic disease, emphasizing genetic mutations. Lastly, the children were not classified according to their pharmacological treatment (beginning and dose), which implies a bias, since it has not been possible to control its action on the dentinal tissue, or the pharmacological deposit of the drug. To date, the effect of systemic bisphosphonates on the process of dental eruption and formation is not clear [43], although alterations in the pathophysiology associated with dentin are described as a rapid deposit of secondary dentin that causes pulpal obliteration in patients with long-term treatments with bisphosphonates [44]. Despite the promising results, adequately designed clinical trials are needed to distinguish the adverse effects of drugs from the dental effects of OI. Another important limitation is that due to the S-shaped trajectory that the dentinal tubules follow through the dentin, not all points could be analyzed perpendicular to its axis, which made it difficult to measure the diameter and tubular density. Knowing that the longitudinal cut of the tooth does not ensure the tubular cut perpendicular to its axis, it was carried out following previous studies on the subject [19,23]; and thanks to the S-shaped path of the dentinal tubules, in many cases it was possible to visualize them as practically transversal. To solve this important bias, the sample was divided for analysis into longitudinal and transversal patterns (it was considered that they could not constitute a single group). Although we consider longitudinal sections to be more difficult to measure, which constitutes a methodological limitation, the data were consistent with those of transverse tubules. In addition, control teeth cut in both patterns were selected to analyze the normality of both types of cuts. One of the main biases that exist in measurements of biological samples is their difficult standardization. In our case, since it is not possible to choose the dental cutting point based on the previously tubular trajectory, we cannot consider the existence of a "perfectly transverse" cut to the tubules, with in many cases beveled samples, which are classified as longitudinal or transversal according to which is closest. This can be considered a bias in terms of the reliability of the measurements, and therefore they were taken as estimates of diameter and tubular density.

Despite the exposed limitations, we believe that our research (with the limitation of an in vitro study) is relevant, since it improves the dental microstructural knowledge of children with OI, generating knowledge about the bone–tooth interrelationship. Clinically, the present results have two applications. The first of them, dental filling, is currently based on a micro-retention obtained from the penetration of resins in the dental tubules forming resin tags, so the alteration of the tubular pattern would affect this type of material and dental adhesive techniques. The dental fragility of patients with OI means that they will need dental treatment on multiple occasions, so research on the function of dental materials in teeth with DGI-I is of vital importance. On the other hand, one of the main concerns that a dentist has when treating this type of teeth is the rapid tooth wear that occurs. Knowing in advance that the dental alteration is not only in relation to tubular architecture, but that there is a mineral deficit, which is more severe in the most severe forms of OI, a dental preventive plan can be carried out aimed at avoiding this loss of dental material and vertical dimension (e.g., in the choice of restorative materials and the preference for a single-unit fixed prosthesis with full coverage versus partial coverage).

The aim is to continue with this line of research, generating a better knowledge of dental microstructure and its interrelation with systemic disease, which would broaden the knowledge of OI. In addition, it would be of great interest to carry out a study in which the relationship between dental microstructure and mineralization with bone mineralization is analyzed, since they are surely closely related, and the bone state could be extrapolated to the dental state and vice versa. In addition, in recent years, the medical treatment of children with OI using bisphosphonates and monoclonal antibodies has become widespread, but their effect on dental hard tissue is in need of further study. Additionally, further studies are needed to test mineral deposition in patients with OI in combination with preventive agents. Historically, fluoride has been the first attempt in dental practice used for preventive purposes [45]; recently, casein phosphopeptide–amorphous calcium phosphate [46] and biomimetic hydroxiapathite [47] have been introduced and showed promising results. Future in vitro and clinical trials are needed to explore this topic.

## 5. Conclusions

With all the above information and its study and detailed analysis, it can be concluded that there is a decrease in the tubular density in the deciduous dentition in children with OI compared to healthy controls in the dentin closest to the pulp, and an increase in the diameter and density tubular in the outermost dentin. The alterations of the dentinal tubules are more pronounced in the most severe phenotypes of systemic disease. The amount of Phosphorus is decreased in the dentin of primary teeth of children with OI compared to the control group.

**Author Contributions:** Conceptualization, A.M.-V., V.V.-G. and M.J.d.N.G.; methodology, A.M.-V.; software, A.M.-V.; validation, A.M.-V., V.V.-G., M.J.d.N.G., J.R.-C. and D.G.-G.; formal analysis, A.M.-V.; investigation, A.M.-V.; resources, A.M.-V.; data curation, A.M.-V.; writing—original draft preparation, A.M.-V.; writing—review and editing, V.V.-G., M.J.d.N.G., J.R.-C. and D.G.-G.; visualization, A.M.-V.; supervision, V.V.-G., M.J.d.N.G., J.R.-C. and D.G.-G.; project administration, V.V.-G. and M.J.d.N.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by Ethics Committee of the "Clínico San Carlos" Hospital (17/326-E Thesis Code) for studies involving humans.

**Informed Consent Statement:** Written informed consent was obtained from all subjects involved in the study. A signed written consent form is required from patients who come to the Faculty of Dentistry to allow their radiographic and/or photographic records to be used for teaching and/or research purposes in accordance with current regulations on data protection and the anonymization of images.

**Data Availability Statement:** The data for this study are available by contacting the corresponding author, A.M.-V. The data are not publicly available due to privacy.

**Acknowledgments:** Thanks to the Department of Clinical Specialties and Conservative Dentistry and Prosthetics of the Faculty of Dentistry.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Sillence, D.O. Osteogenesis imperfecta nosology and genetics. *Ann. N. Y. Acad. Sci.* **1988**, *543*, 1–15. [CrossRef]
2. Van Dijk, F.S.; Sillence, D.O. Osteogenesis imperfecta: Clinical diagnosis, nomenclature and severity assessment. *Am. J. Med. Genet. Part A* **2014**, *164*, 1470–1481. [CrossRef]
3. Forlino, A.; Marini, J.C. Osteogenesis imperfecta. *Lancet* **2016**, *387*, 1657–1671. [CrossRef]
4. Gutiérrez-Díez, M.P.; Molina Gutiérrez, M.A.; Prieto Tato, L.; Parra García, J.I.; Bueno Sánchez, A.M. Osteogénesis imperfecta: Nuevas perspectivas. *Rev. Esp. Endocrinol. Pediatr.* **2013**, *4*, 75–85.
5. Huber, M.A. Osteogenesis imperfecta. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2007**, *103*, 314–320. [CrossRef]
6. Sillence, D.; Senn, A.; Danks, D.M. Genetic heterogeneity in osteogenesis imperfecta. *J. Med. Genet.* **1979**, *16*, 101–116. [CrossRef] [PubMed]
7. Shields, E.D.; Bixler, D.; El-Kafrawy, A.M. A proposed classification for heritable human dentine defects with a description of a new entity. *Arch. Oral Biol.* **1973**, *18*, 543-IN7. [CrossRef] [PubMed]
8. Lin, H.; Chuang, C.; Su, Y.; Chen, M.; Chiu, H.; Niu, D.; Lin, S. Genotype and phenotype analysis of Taiwanese patients with osteogenesis imperfecta. *Orphanet J. Rare Dis.* **2015**, *10*, 152. [CrossRef]
9. Pallos, D.; Hart, P.S.; Cortelli, J.R.; Vian, S.; Wright, J.T.; Korkko, J.; Brunoni, D.; Hart, T.C. Novel COL1A1 mutation (G599C) associated with mild osteogenesis imperfecta and dentinogenesis imperfecta. *Arch. Oral Biol.* **2001**, *46*, 459–470. [CrossRef] [PubMed]
10. Teixeira, C.S.; Felippe, M.C.S.; Felippe, W.T.; Silva-Sousa, Y.T.C.; Sousa-Neto, M.D. The role of dentists in diagnosing osteogenesis imperfecta in patients with dentinogenesis imperfecta. *J. Am. Dent. Assoc.* **2008**, *139*, 906–914. [CrossRef]
11. Majorana, A.; Bardellini, E.; Brunelli, P.C.; Lacaita, M.; Cazzolla, A.P.; Favia, G. Dentinogenesis imperfecta in children with osteogenesis imperfecta: A clinical and ultrastructural study. *Int. J. Paediatr. Dent.* **2010**, *20*, 112–118. [CrossRef] [PubMed]
12. O'connell, A.C.; Marini, J.C. Evaluation of oral problems in an osteogenesis imperfecta population. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **1999**, *87*, 189–196. [CrossRef] [PubMed]
13. Glorieux, F.H. Osteogenesis imperfecta. *Best Pract. Res. Clin. Rheumatol.* **2008**, *22*, 85–100. [CrossRef]

14. Hall, R.K.; Manière, M.; Palamara, J.; Hemmerle, J. Odontoblast dysfunction in osteogenesis imperfecta: An LM, SEM, and ultrastructural study. *Connect. Tissue Res.* **2002**, *43*, 401–405. [CrossRef]
15. Barron, M.J.; McDonnell, S.T.; MacKie, I.; Dixon, M.J. Hereditary dentine disorders: Dentinogenesis imperfecta and dentine dysplasia. *Orphanet J. Rare Dis.* **2008**, *3*, 31. [CrossRef]
16. Fratzl-Zelman, N.; Misof, B.M.; Roschger, P.; Klaushofer, K. Klassifikation der Osteogenesis imperfecta. *Wien. Med. Wochenschr.* **2015**, *165*, 264–270. [CrossRef] [PubMed]
17. Hoyer-Kuhn, H.; Netzer, C.; Semler, O. Osteogenesis imperfecta: Update zu Pathophysiology und Therapie. *Wien. Med. Wochenschr.* **2015**, *165*, 278–284. [CrossRef] [PubMed]
18. Malmgren, B.; Norgren, S. Dental aberrations in children and adolescents with osteogenesis imperfecta. *Acta Odontol. Scand.* **2002**, *60*, 65–71. [CrossRef]
19. Lygidakis, N.A.; Smith, R.; Oulis, C.J. Scanning electron microscopy of teeth in osteogenesis imperfecta type I. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **1996**, *81*, 567–572. [CrossRef]
20. Martín-Vacas, A.; de Nova, M.J.; Sagastizabal, B.; García-Barbero, Á.E.; Vera-González, V. Morphological Study of Dental Structure in Dentinogenesis Imperfecta Type I with Scanning Electron Microscopy. *Healthcare* **2022**, *10*, 1453. [CrossRef] [PubMed]
21. Bencharit, S.; Border, M.B.; Mack, C.R.; Byrd, W.C.; Wright, J.T. Full-mouth rehabilitation for a patient with dentinogenesis imperfecta: A clinical report. *J. Oral Implantol.* **2014**, *40*, 593–600. [CrossRef]
22. Malmgren, B.; Lindskog, S. Assessment of dysplastic dentin in osteogenesis imperfecta and dentinogenesis imperfecta. *Acta Odontol. Scand.* **2003**, *61*, 72–80. [CrossRef] [PubMed]
23. Lindau, B.; Dietz, W.; Lundgren, T.; Storhaug, K.; Noren, J.G. Discrimination of morphological findings in dentine from osteogenesis imperfecta patients using combinations of polarized light microscopy, microradiograhpy and scanning electron microscopy. *Int. J. Paediatr. Dent.* **1999**, *9*, 253–261. [CrossRef] [PubMed]
24. Lindau, B.M.; Dietz, W.; Hoyer, I.; Lundgren, T.; Storhaug, K.; Noren, J.G. Morphology of dental enamel and dentine–enamel junction in osteogenesis imperfecta. *Int. J. Paediatr. Dent.* **1999**, *9*, 13–21. [CrossRef] [PubMed]
25. Kantaputra, P.N.; Sirirungruangsarn, Y.; Intachai, W.; Ngamphiw, C.; Tongsima, S.; Dejkhamron, P. Osteogenesis imperfecta with ectopic mineralizations in dentin and cementum and a COL1A2 mutation. *J. Hum. Genet.* **2018**, *63*, 811–820. [CrossRef]
26. Gasga, J.R. Sample Preparation of Human Tooth Samples for Observation by Light Microscopy, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). *Acta Microscópica* **2020**, *29*, 121–131.
27. Waltimo, J.; Ojanotko-Harri, A.; Lukinmaa, P. Mild forms of dentinogenesis imperfecta in association with osteogenesis imperfecta as characterized by light and transmission electron microscopy. *J. Oral Pathol. Med.* **1996**, *25*, 256–264. [CrossRef]
28. De Coster, P.J.; Cornelissen, M.; De Paepe, A.; Martens, L.C.; Vral, A. Abnormal dentin structure in two novel gene mutations [COL1A1, Arg134Cys] and [ADAMTS2, Trp795-to-ter] causing rare type I collagen disorders. *Arch. Oral Biol.* **2007**, *52*, 101–109. [CrossRef]
29. Levin, L.S.; Brady, J.M.; Melnick, M.; Optiz, J.M. Scanning electron microscopy of teeth in dominant osteogenesis imperfecta: Support for genetic heterogeneity. *Am. J. Med. Genet.* **1980**, *5*, 189–199. [CrossRef]
30. Waltimo, J. Hyperfibers and vesicles in dentin matrix in dentinogenesis imperfecta (Dl) associated with osteogenesis imperfecta (01). *J. Oral Pathol. Med.* **1994**, *23*, 389. [CrossRef] [PubMed]
31. Sumikawa, D.A.; Marshall, G.W.; Gee, L.; Marshall, S.J. Microstructure of primary tooth dentin. *Pediatr. Dent.* **1999**, *21*, 439–444. [PubMed]
32. De Ferraris, M.E.G.; Muñoz, A.C. *Histologia, Embriologia E Ingenieria Tisular*; Médica Panamericana: Buenos Aires, Argentina, 2009.
33. Berkovitz, B.; Holland, G.R.; Moxham, B.J.; Holland, G.R.; Moxham, B.J. *Oral Anatomy, Histology and Embryology*; 4th International Edition; Mosby: London, UK, 2009; Volume 4.
34. Nanci, A. *Ten Cate's Oral Histology: Development, Structure, and Function*; Elsevier Health Sciences: Philadelphia, PA, USA, 2007.
35. Koutsi, V.; Noonan, R.G.; Horner, J.A.; Simpson, M.D.; Matthews, W.G.; Pashley, D.H. The effect of dentin depth on the permeability and ultrastructure of primary molars. *Pediatr. Dent.* **1994**, *16*, 29. [PubMed]
36. Schilke, R.; Lisson, J.A.; Bauß, O.; Geurtsen, W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. *Arch. Oral Biol.* **2000**, *45*, 355–361. [CrossRef]
37. Ruschel, H.C.; Chevitarese, O. Density and diameter of dentinal tubules of first and second primary human molars-comparative scanning electron microscopy study. *J. Clin. Pediatr. Dent.* **2002**, *26*, 297–304. [CrossRef] [PubMed]
38. Lenzi, T.L.; Guglielmi, C.d.A.B.; Arana-Chavez, V.E.; Raggio, D.P. Tubule density and diameter in coronal dentin from primary and permanent human teeth. *Microsc. Microanal.* **2013**, *19*, 1445–1449. [CrossRef]
39. Lakomaa, E.; Rytömaa, I. Mineral composition of enamel and dentin of primary and permanent teeth in Finland. *Eur. J. Oral Sci.* **1977**, *85*, 89–95. [CrossRef]
40. Derise, N.L.; Ritchey, S.J.; Furr, A.K. Mineral composition of normal human enamel and dentin and the relation of composition to dental caries: I. Macrominerals and comparison of methods of analyses. *J. Dent. Res.* **1974**, *53*, 847–852. [CrossRef]
41. Saiki, M.; Adachi, L.K.; Adachi, E.M. Elemental comparison in sound and carious human teeth by instrumental neutron activation analysis. *J. Radioanal. Nucl.* **2009**, *282*, 29–32. [CrossRef]
42. Pragnère, S.; Auregan, J.C.; Bosser, C.; Linglart, A.; Bensidhoum, M.; Hoc, T.; Nouguier-Lehon, C.; Chaussain, C. Human dentin characteristics of patients with osteogenesis imperfecta: Insights into collagen-based biomaterials. *Acta Biomater.* **2021**, *119*, 259–267. [CrossRef]

43. Soares, A.P.; do Espírito Santo, R.F.; Line, S.R.P.; Pinto, M.d.G.F.; de Moura Santos, P.; Toralles, M.B.P.; do Espírito Santo, A.R. Bisphosphonates: Pharmacokinetics, bioavailability, mechanisms of action, clinical applications in children, and effects on tooth development. *Environ. Toxicol. Pharmacol.* **2016**, *42*, 212–217. [CrossRef]
44. Marçal, F.F.; Ribeiro, E.M.; Costa, F.W.G.; Fonteles, C.S.R.; Teles, G.S.; de Barros Silva, P.G.; Junior, C.M.C.; Ribeiro, T.R. Dental alterations on panoramic radiographs of patients with osteogenesis imperfecta in relation to clinical diagnosis, severity, and bisphosphonate regimen aspects: A STROBE-compliant case-control study. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* **2019**, *128*, 621–630. [CrossRef]
45. Zampetti, P.; Scribante, A. Historical and bibliometric notes on the use of fluoride in caries prevention. *Eur. J. Paediatr. Dent.* **2020**, *21*, 148–152. [PubMed]
46. Fallahzadeh, F.; Heidari, S.; Najafi, F.; Hajihasani, M.; Noshiri, N.; Nazari, N.F. Efficacy of a Novel Bioactive Glass-Polymer Composite for Enamel Remineralization following Erosive Challenge. *Int. J. Dent.* **2022**, *2022*, 6539671. [CrossRef] [PubMed]
47. Butera, A.; Pascadopoli, M.; Pellegrini, M.; Trapani, B.; Gallo, S.; Radu, M.; Scribante, A. Biomimetic hydroxyapatite paste for molar–incisor hypomineralization: A randomized clinical trial. *Oral Dis.* **2023**, *29*, 2789–2798. [CrossRef] [PubMed]