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SEM and FT-MIR Analysis of Human Demineralized Dentin Matrix: An In Vitro Study

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Abstract: Recently, the demineralized dentin matrix has been suggested as an alternative material to autologous bone grafts and xenografts for clinical purposes. The aim of this study was to investigate the effect of different times of demineralization on the chemical composition and the surface morphology of dentinal particles. Extracted teeth were ground and divided into 5 groups based on demineralization time (T0 = 0 min, T2 = 2 min, T5 = 5 min, T10 = 10 min, and T60 = 60 min) with 12% EDTA. The analysis was performed using Fourier-Transform Mid-Infrared spectroscopy (FT-MIR) and Scanning Electron Microscopy (SEM) ($p < 0.05$). The FT-MIR analysis showed a progressive reduction of the concentration of both PO_4^{3-} and CO_3^{2-} in the specimens (T0 > T2 > T5 > T10 > T60). On the contrary, the organic (protein) component did not undergo any change. The SEM examination showed that increasing the times of demineralization resulted in a smoother surface of the dentin particles and a higher number of dentinal tubules.

Keywords: demineralized dentin matrix; human demineralized dentin matrix; human; bone graft; FT-MIR; SEM

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1. Introduction

After tooth extraction, hard and soft tissue withstand remodeling processes. Alveolar ridge undergoes resorption, mostly in the horizontal but also in the vertical dimension [1]. Several studies analyzed the changes after tooth extraction [2]. The majority of horizontal and vertical changes take place during the first 3–6 months after tooth extraction and continue through the first year [3]. In fact, the horizontal bone loss is higher on the buccal side of the alveolar ridge than on the lingual/palatal side. On the other hand, the vertical resorption is minor and mainly on the buccal aspect of the alveolar ridge. This volumetric contraction of the alveolar ridge may jeopardize an appropriate implant-supported prosthetic rehabilitation.

Several surgical techniques have been developed to reduce or, at least, minimize the changes of soft and hard tissue following tooth loss. The main alveolar ridge preservation techniques concern on soft-tissue preservation, hard-tissue preservation (guided bone regeneration) and the combination of soft tissue and hard tissue preservation (socket seal technique) [4]. Moreover, different regenerative techniques have been tested: the use of bone grafts alone; barrier membranes alone, either resorbable or not; the combination of barrier membranes and bone graft. Metanalyses have shown that alveolar ridge preservation techniques are effective in significantly reducing vertical and horizontal alveolar ridge contraction [5,6]. Also Troiano et al. [7] reported positive results in the use of bone grafting and resorbable membrane compared with spontaneous healing. Autologous bone represents the ideal graft material due to its osteoinductive and osteoconductive

properties [8]. Nevertheless, its limits are the small amount of bone graft available, the morbidity of the donor area, and the risk of resorption of the bone graft itself. The research has led to developing alternatives, such as allogenic grafts, alloplastic grafts, and xenogenic grafts [9].

In the past decades, the use of demineralized dentin matrix (DDM) as a potential bone substitute has been proposed. Dentin consists of (i) 70% inorganic component (hydroxyapatite, tricalcium phosphate, octacalcium phosphate, and amorphous calcium phosphate); (ii) 20% organic component (collagen I 90%, collagen III and V in small quantities, and non-collagenic proteins); (iii) 10% water [10]. Its composition is, therefore, similar to that of bone tissue. Dentin also contains various growth factors, such as Fibroblast Growth Factors -2 (FGF-2), transforming growth factors-β1 (TGF-β1), insulin growth factor-1 (IGF-1) and, above all, bone morphogenetic proteins (BMPs) involved in the osteogenesis process [10].

Through demineralization, dentin can release the growth factors [11] and consequently express its osteoinductive and osteoconductive properties [12]. Thereby acting as a scaffold, dentin promotes the formation of bone tissue (osteoconduction), but at the same time, it releases the growth factors that promote the formation of bone (osteoinduction). Moreover, different in vivo studies have reported encouraging results [13–15] that led to this material being considered as a possible alternative to autologous bone grafts [16].

It has been shown that the preparation procedure, the shape, and the size of the dentin particles can influence dentin's osteoconductive and osteoinductive properties [17]. Excessive demineralization can damage the structure of the dentin and negatively affect the composition and function of growth factors; on the other hand, a reduced demineralization produces a scaffold with osteoconductive and osteoinductive properties [18].

The aims of this study were: (i) to examine the changes in the chemical composition of dentin particles using Fourier-transform mid-infrared spectroscopy (FT-MIR) analysis after different exposure times to demineralizing agent; (ii) to evaluate the surface morphology of the dentin particles by Scanning Electron Microscopy (SEM) analysis after different exposure times to demineralizing agent.

2. Materials and Methods

2.1. Experimental Design and Specimen Preparation

For this study, we used extracted teeth with unfavorable prognoses with the consent of patients. The criteria for exclusion were: (i) teeth with carious lesions; (ii) teeth with root canal treatment. After extraction of the specimens, the enamel and root cementum were removed with a dental drill (Figure 1a). Next, each tooth was washed with physiological solution, rinsed with an air-spray tool, and stored at −20 °C. The teeth were placed in a sterile container (Figure 1b) and ground using a Smart Dentin Grinder (KometaBio Inc., Cresskill, NJ, USA) for 3 s (Figure 1c). Then, the dentinal powder was sieved to distinguish two specimens with different grain sizes: the first with granules of smaller sizes (<300μm) (A); and the second with granules of sizes between 300 μm and 1200 μm (B). The specimens were collected in sterile containers (Figure 1d,e). In this study, we used only the second specimen (B), while the first was excluded (specimen A).

Following the instructions of the machine producer, the dentinal powder was immersed in basic alcohol for 10 min in a sterile glass container. The basic alcohol was composed of 0.5 M NaOH and 20% ethanol. Next, the dentinal powder was picked up and washed two times with sterile phosphate-buffered saline solution (3 min for each wash) (Figure 1f). The dentinal powder was washed with a physiological solution and rinsed as much as possible.

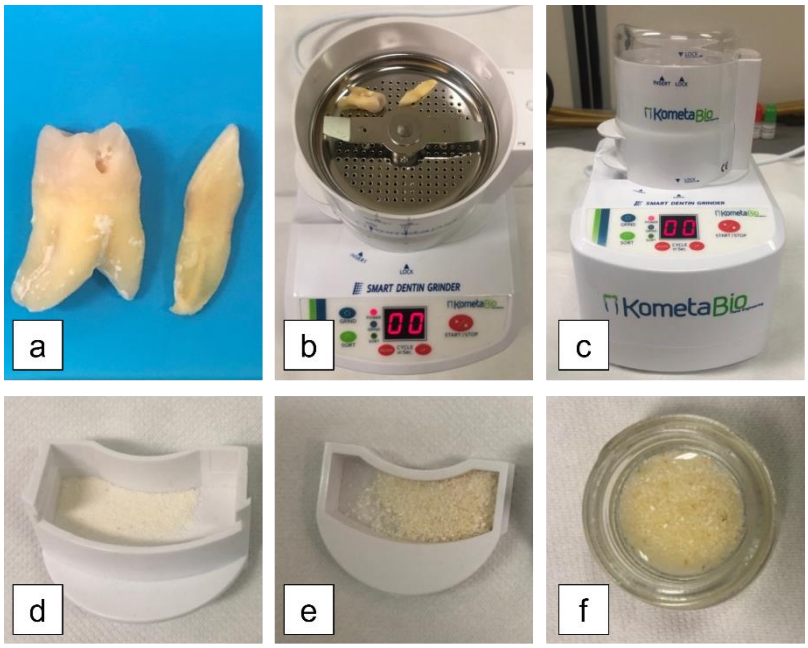


Figure 1. Specimen preparation: (**a**) Example of the final specimens after enamel and cementum removal with a dental drill; (**b**) specimens in the sterile trituration chamber; (**c**) detail of Smart Dentin Grinder machine (KometaBio Inc., Cresskill, NJ, USA); (**d**) ground dentin with particle size less than 300 μm; (**e**) ground dentin with particle size between 300 μm and 1200 μm; (**f**) dentin particulate immersed in a sterile container with sterile saline solution.

The dentinal powder was randomly divided into five groups (0.075 g of dentin each) based on the time of demineralization (Table 1): T2, 2 min; T5, 5 min; T10, 10 min; T60, 60 min. T0 was not-demineralized and was considered as the control group.

12 %EDTA was used to demineralize the dentin. Specifically, 1.7 g EDTA disodium salt was dissolved in 10mL distilled water at 25 °C. A saturated solution was obtained, from which only the supernatant was taken. Through this process, we obtained EDTA at 12%. The choice of this concentration was made according to previous articles published in the literature [12,17]. An amount of 1.1 mL EDTA was applied for the established time using a specific tool (Vortex). Once the process was completed, EDTA was removed, and the specimens were stored at −20 °C. Each specimen was washed two times with a physiologic solution (400 μL) to stop the demineralizing process. Next, the physiologic solution was aspirated.

Table 1. Group nomenclature based on demineralization process.

Nomenclature	Demineralization Process
T0	not-demineralized
T2	2 min in 12% EDTA dentin particles
T5	5 min in 12% EDTA dentin particles
T10	10 min in 12% EDTA dentin particles
T60	60 min in 12% EDTA dentin particles

The water had to be removed from the specimens to allow the spectrophotometry analysis. This process was completed by putting the specimens in a centrifuge (SpeedVac Concentrator, Savant SPD111V) (Figure 2a) for 30 min. The specimens were also weighed at different times: (i) ground and centrifuged dentin, before the demineralization process (D1); (ii) ground and demineralized dentin (D2); (iii) ground and centrifuged dentin after demineralization process (D3).

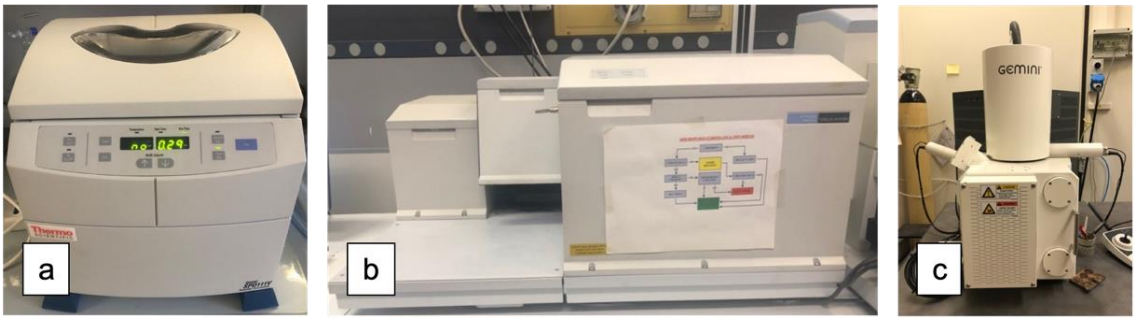


Figure 2. Instruments used in this study. (**a**) SpeedVac Concentrator, Savant SPD111V was used for the elimination of residual water; (**b**) FT-IR Perkin Elmer Spectrum One. Acquisition range 4000–450 cm^{-1}; (**c**) scanning electron microscope (Supra 40, Zeiss).

2.2. FT-MIR Analysis

FT-MIR analysis was carried out using the FT-IR Perkin Elmer Spectrum One (Figure 2b) (Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy). The analysis of all specimens was performed in reflection UATR mode (Attenuate Total Reflection) with a range between 4000–600 cm^{-1} (spectral resolution 4 cm^{-1}, 16 scans). The bands used for this study were: band 1021 cm^{-1}, which indicated the percentage of PO_4^{3-}; band 1649 cm^{-1}, which corresponded to the protein component and was performed to determine the percentage of mineralization of each specimen; and band 872 cm^{-1}, which reflected the CO_3^{2-} percentage.

2.3. SEM Analysis

The specimens were gold-coated and analyzed through SEM (Department of Materials, Environmental Science and Urban Planning, Polytechnic University of Marche, Ancona, Italy). The superficial morphology of each specimen was evaluated through detector SE2 (Figure 2c) at different magnifications: (i) 400×; (ii) 2000×; (iii) 8000×.

2.4. Statistical Analysis

Statistical analysis of data included analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

3. Results

3.1. Weight Analysis of the Specimens

Table 2 shows the average weight of D1, D2 and D3.

Table 2. The weights of the specimens (expressed in g) in D1 (triturated and centrifuged dentin), D2 (triturated and demineralized dentin), and D3 (triturated, demineralized, and centrifuged dentin).

Specimen	T2	T5	T10	T60
D1	0.075 g	0.075 g	0.075 g	0.075 g
D2	0.093 g	0.081 g	0.051 g	0.024 g
D3	0.061 g	0.057 g	0.034 g	0.014 g

3.2. FT-MIR

MIR spectra were acquired for each specimen in the 4000–600 cm^{-1} spectral range, and NIR in the 10000–4000 cm^{-1} range. The main MIR absorption bands considered in this study are shown in Figure 3, which shows the spectrum of the dentin not subjected to demineralization (T0). Based on previous studies [19–21], the main absorption bands analyzed in this study were: (i) ~1649 cm^{-1} band corresponding to the protein component of dentin (band A); (ii) ~1021 cm^{-1} band corresponding to the phosphate ion PO_4^{3-} (band B); (iii) ~872 cm^{-1} band corresponding to the stretching of the carbonate CO_3^{2-} (band C).

The variations in the MIR spectral profiles of the analyzed specimens (T0, T2, T5, T10, T60) were acquired in the spectral range 4000–600 cm^{-1}. Then, the spectra were normalized compared to the 1649 cm^{-1} band, which corresponded to the protein component of dentin, and which remained constant even after the demineralization treatment. Through this procedure, it was possible to observe the trends of the PO_4^{3-} and CO_3^{2-} groups (Figure 4).

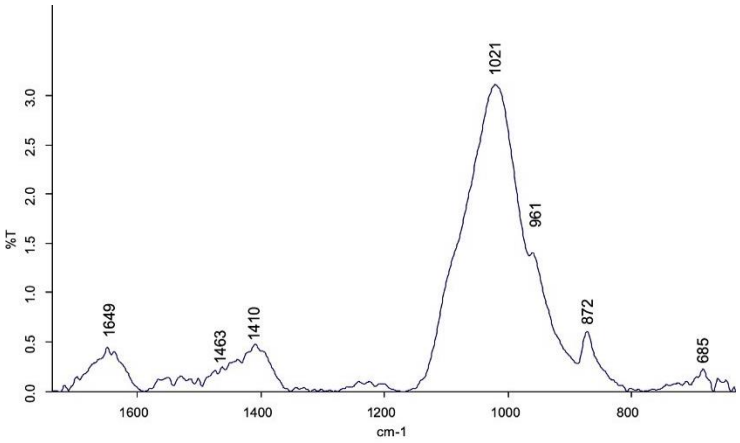


Figure 3. Spectrum acquired through FT-MIR analysis of dentin particles not subjected to demineralization with 12% EDTA.

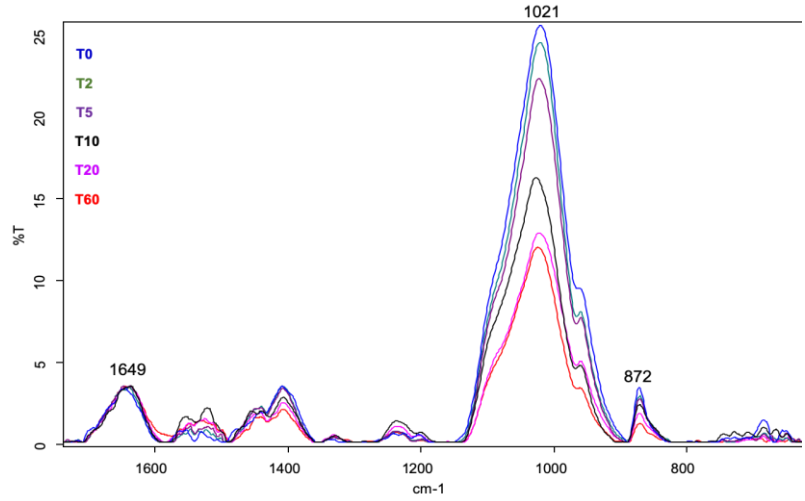
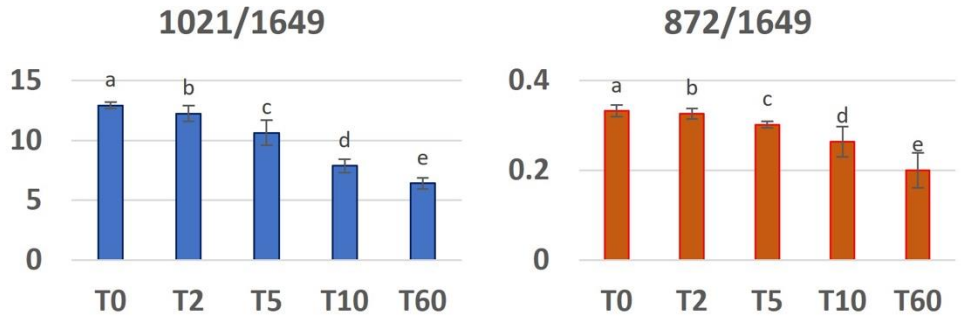


Figure 4. Spectra of the analyzed specimens normalized at the level of the 1649 cm^{-1} band, which corresponded to the protein component of dentin. Reductions were observed in the band at 1021 cm^{-1}, which resembled the phosphate group, and at 872 cm^{-1}, which corresponded to the carbonate group.

The concentrations of the inorganic components of dentin (PO_4^{3-} and CO_3^{2-} groups) decreased with increasing exposure time to the demineralizing agent. The amount of the mineralized component of the dentin was greater in T0, followed by T2, T5, T10. The T60

specimens were subject to the longest time of demineralization and showed the least amount of mineralized component.

The ratio between band B (1021 cm^{-1}), which described the percentage of PO_4^{3-}, and band A (1649 cm^{-1}), which corresponded to the protein component, was measured to determine the percentage of mineralization of each specimen (Figure 5a). Band C (872 cm^{-1}), which reflected the CO_3^{2-} percentage, was also compared with band A (1649 cm^{-1}), which corresponded to the protein component (Figure 5b).



(**a**) (**b**)

Figure 5. FT-MIR Analysis. (**a**) The ratio between band B (1021 cm^{-1}), which corresponded to the PO_4^{3-} group, and band A (1649 cm^{-1}), which reflected the protein component; (**b**) the ratio between band C (872 cm^{-1}), which represented the CO_3^{2-} group, and band A (1649 cm^{-1}), which corresponded to the protein component. Different letters represent statistically significant differences ($p < 0.05$).

At T0, dentin was not treated with 12% EDTA, so it was considered as the control group, assuming 100% mineralization. Next, we calculated the quantity of PO_4^{3-} and CO_3^{2-} as a percentage of each specimen (Figure 6).

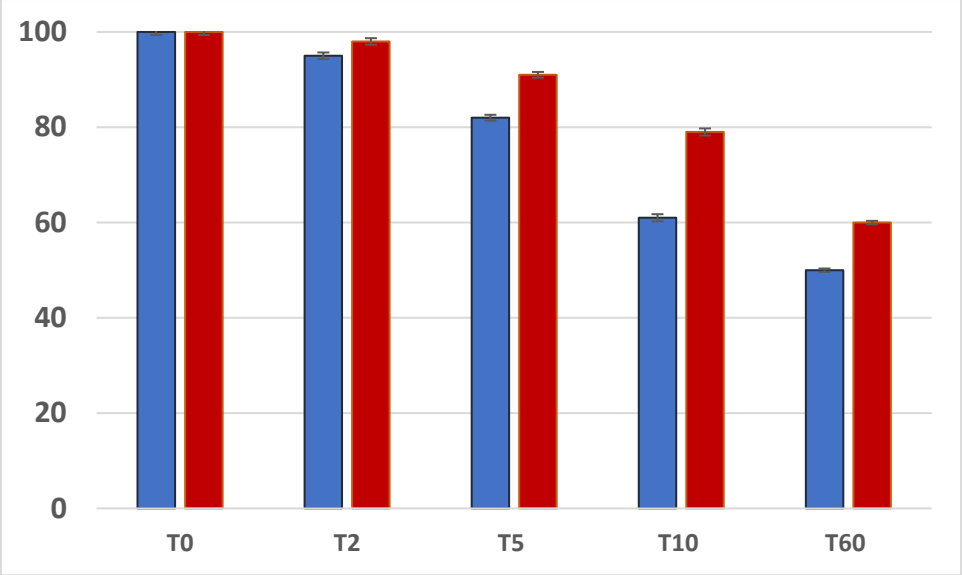


Figure 6. The graph represents the percentage of mineralization of the analyzed specimens after different exposure times. The blue expresses the quantity (%) of the PO_4^{3-} group; the orange describes the quantity (%) of the CO_3^{2-} group.

3.3. SEM

Finally, in this study, an SEM morphological evaluation of the specimens was performed (Figure 7). For each specimen, the obtained microanalysis was derived from the

average of the results obtained on the most representative area. The voltage used was 25 kW, while the focal length was 15 mm.

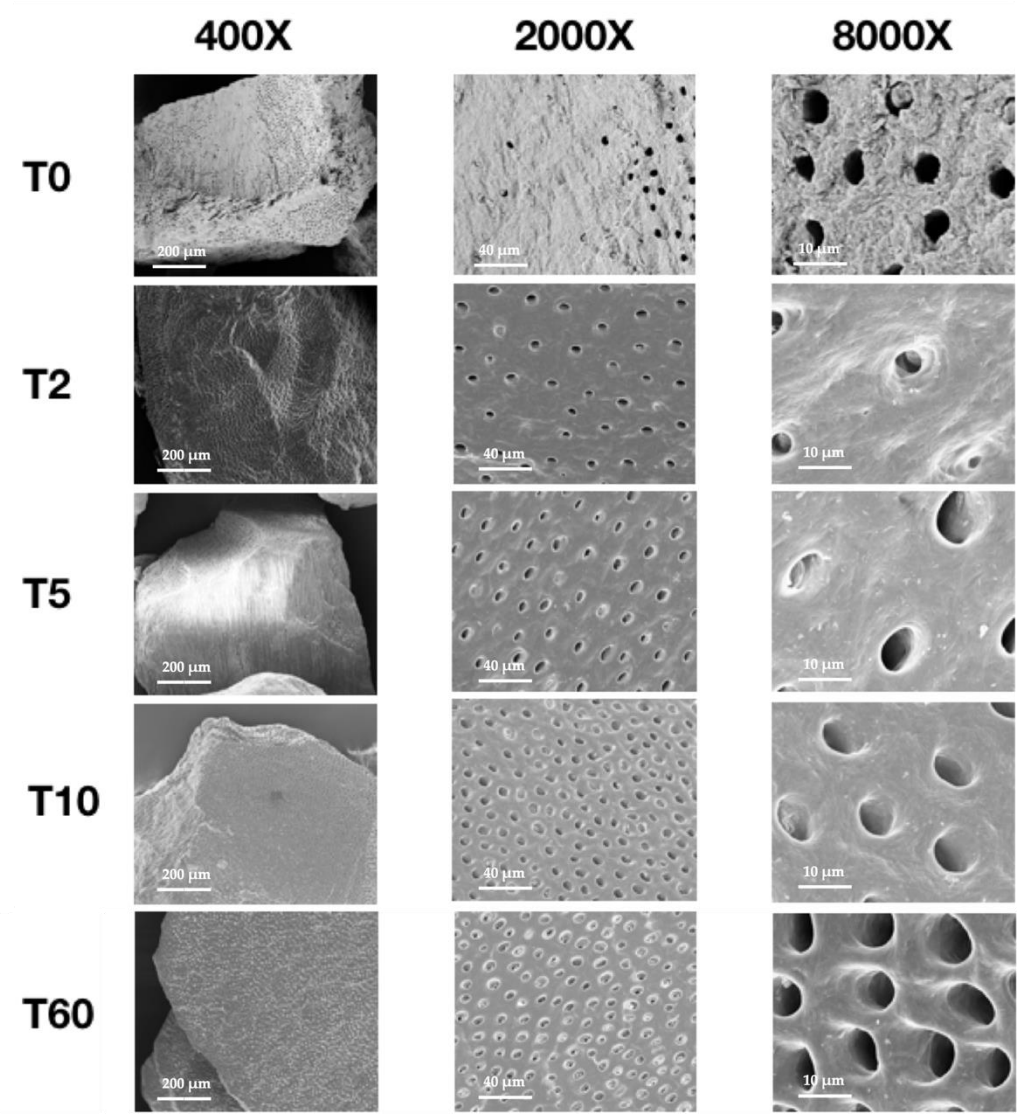


Figure 7. The acquired SEM images of the specimens T0, T2, T5, T10, T60 at different magnifications: 400×, 2000×, and 8000×.

The images were acquired at different magnifications (400×, 2000×, and 8000×). This analysis allowed us to investigate the form and number of dentinal tubules exposed on the surface of the specimen. At 2000× magnification, it was seen that the number of the dentinal tubules in the same surface area increased T0 < T2 < T5 < T10 < T60.

Comparing the image at 8000× magnification of T0 with the one of T60, we observed a difference at the surface of the specimens. In the T0 specimens, the surface of the dentin was rough and non-homogeneous, while the dentin surface of the T60 specimen was smoother and more homogeneous. According to these results, it was assumed that the surface roughness tended to decrease with longer exposure to the demineralizing agent.

4. Discussion

An ideal bone graft should be biocompatible, biomechanically stable, capable of degrading over a certain time, and exhibit osteoconductive, osteogenic, and osteoconductive properties [22]. Although the gold standard is exemplified by autologous bone, the

limited amount of bone available, the morbidity of the donor site, and the high rate of resorption affect its use. Therefore, alternative materials for autologous bone grafts are required.

In recent years, it has been proposed that DDM be used as a potential bone graft. In Japan and Korea, this type of graft is widely used, as demonstrated by the number of scientific works published in the literature by scientific authors [23]. It has been shown that the preparation process, the size, and shape of the dentin particles seem to influence their osteoinductive and osteoconductive properties [17].

In summary, the concentration of the phosphate group (inorganic component of dentin, corresponding to the spectral peak at 1021 cm^{-1}) and the carbonate group (inorganic component of dentin, corresponding to the spectral peak at 872 cm^{-1}) decreased with increasing time of demineralization (T0 > T2 > T5 > T10 > T60). In addition, with increasing demineralization time, the number of exposed dentinal tubules in the same surface area increased, and the particles become more homogeneous and smoother.

In the present study, the enamel and the cementum were removed from the extracted teeth. In fact, the hydroxyapatite in the enamel is structured as highly crystalline calcium phosphate, while the dentin contains hydroxyapatite in low crystalline calcium phosphate form. In the first case, the high crystalline content is not easy to decompose by osteoclasts. Consequently, the resorption rate is slow, and the material's osteoconductivity is reduced. On the contrary, hydroxyapatite in dentin has a low crystalline structure, and this makes its resorption easier [11]. Bone tissues also contain low crystalline apatite. Recently, Elfana et al. [24] performed a randomized clinical trial comparing autologous whole tooth grafts and the autologous demineralized dentin grafts. The histological results showed a higher amount of newly formed bone and a smaller number of remnant grafts in the autologous demineralized dentin grafts group. The authors hypothesized that in the autologous demineralized dentin grafts group, the lower mineral content made particle degradation faster than that in the autologous whole tooth grafts group, and this allowed the release of growth factors earlier.

Regarding the size of the particles, a clear consensus has not yet been reached on which precise size is most suitable for bone grafts. Shapoff et al. [25] stated that the particle size of bone grafts should be between 100 μm and 300 μm. Nam et al. [26] conducted an in vivo study testing the new bone formation capabilities of DDM grafts with different densities and particle sizes. The histomorphometric analysis demonstrated the superiority of the specimens with grafting particles sized between 250 μm and 1000 μm and spaces of 200 μm between the particles compared to the results obtained with grafts with larger particles (1000–2000 μm). Koga et al. [12] reported better results in terms of new bone formation with particle sizes between 1200 μm and 800 μm compared to those obtained with smaller particle sizes. The authors also observed that the smaller particles underwent faster resorption than the larger ones. Therefore, it was suggested that the larger-sized particles (1200–800 μm) offer a greater surface area than those of smaller sizes (180–212 μm and 425–600 μm) for the adhesion of osteoprogenitor cells and osteoblasts. In addition, the adhesion of these cells could prevent the absorption of DDM particles and start the formation of new bone. For these reasons, in our study, demineralized dentin particles ranging in size from 300 μm to 1200 μm were used, excluding the smaller-sized particles (<300 μm).

Many studies have reported different tooth processing methods. Generally, there are four main categories [17]: (i) extraction of non-collagenic proteins from dentin; (ii) demineralization; (iii) elimination of the organic matrix (denaturation); (iv) use of tooth particles without modification. Denaturation is a little-used method because it eliminates the proteins in the matrix, including the growth factors responsible for the osteoinductive capacity of the dentin itself. Currently, the most used protocol is demineralization, as demonstrated by a large number of studies in the literature. The most used demineralizing agents are: (i) EDTA [17,24,27]; (ii) HNO_3 [12,27]; and (iii) HCl [28–30].

Demineralization does not affect the organic component of the dentin or damage the growth factors contained therein. This process increases the osteoinductivity of the dentin particles, since it promotes the release of growth factors [11], favors the adhesion of osteoblasts through the exposure of collagen fibers [12], and reduces dentin's antigenicity. Demineralization is necessary because crystalline hydroxyapatite inhibits the release of growth factors, such as BMPs [11]. It has been observed that the amount of time during which a demineralizing agent acts influences the characteristics of dentin. An excessive demineralization can damage the dentin structure and adversely affect the composition and function of odontogenic factors. On the other hand, a mild demineralization produces a scaffold with poor osteoinductive capabilities [18].

Tanoue et al. [27] performed an FIB/SEM analysis of the demineralized (HNO_3 2%) dentin matrix grafted in a rat calvaria bone defect model. This method allowed the 3D reconstruction of the interface between the implanted dentin particles and the surrounding bone. The diameters of the exposed dental tubules averaged 3 μm. Mesenchymal cells, such as osteoblasts and bone tissue cells, are between 10–20 μm in size, while osteoclasts are 20–100 μm. The results of this study showed that osteocytes surrounded the grafted dentin particles, forming a network on their surfaces. In addition, cytoplasmatic extensions of osteocytes were observed in the dentinal tubules contained within the dentin particles. The authors also hypothesized a possible biological sequence of events that occurs when demineralized dentin particles are grafted into the recipient site. The release of BMPs induces mesenchymal cells to differentiate into osteoblasts, as also shown by the immunohistochemical analysis of de Oliviera et al. [31]. These cells produce a matrix that undergoes mineralization and forms new bone. At this point, the osteoblasts differentiate into osteocytes, which adhere to the surface of the demineralized dentin particles, forming a network on their surface. Cytoplasmatic extensions from the network spread into the dentinal tubules contained within the dentin particles. Afterward, the dentin particles will be reabsorbed and replaced by new bone, as confirmed by Kim et al. [13].

BMPs belong to the large family of TGF-β. Urist [32] was the first to describe their biological activity. In the following years, considerable efforts were made to isolate these growth factors and study both their in vitro and in vivo features. The BMPs promote the differentiation of mesenchymal cells into osteoblasts and chondroblasts [33], participating in the development of bone and cartilage [34], rather than the formation of dental hard tissues [35]. Bessho et al. [36,37] showed that BMPs obtained from demineralized dentin have osteoinductive properties similar to those derived from bone tissue. The BMPs induced new bone formation through endochondral (indirect) and intramembranous (direct) ossification, as shown by the histological findings of Murata et al. [30].

In our study, the dentin particles underwent a demineralization process with 12% EDTA. This agent is widely used in endodontics as a chelating agent for the enlargement of the canals and the removal of the smear layer. The longer the EDTA works on dentin, the more evident its effects are on dentin, as demonstrated by the release of phosphorus [38]. The specimens in our study were divided based on the exposure time of EDTA (0 min; 2 min; 5 min; 10 min; 60 min). The effect of the demineralizing agent was demonstrated by the analysis of the weight of the specimens, the FT-MIR analysis, and also SEM.

The weight of each specimen was lower after the demineralization process. One of the most used methods to confirm demineralization is SEM [18]. The results of our SEM analysis showed that the longer the EDTA was allowed to work, the less rough, or smoother, the surfaces of the particles became. Furthermore, it was observed that the longer the duration of demineralization, the greater the number of dentinal tubules exposed on the same surface area of the particles.

Koga et al. [12] conducted an in vivo study comparing non-demineralized, partially demineralized, and completely demineralized dentin specimens. Similarly to our study, the SEM analysis showed that the surfaces of the demineralized dentin specimens were smoother while those of the non-demineralized dentin were rougher. Interestingly, the osteoblasts adhered only to the surfaces of demineralized dentin but not to those of non-

demineralized dentin. The authors hypothesized that the exposure of collagen fibers following demineralization could promote the adhesion of osteoblasts. The specimens of partially and completely demineralized dentin matrix, above all, showed greater osteogenic power than the non-demineralized ones.

Similar SEM results were achieved by Tabatabei et al. [18] performed a SEM analysis of dentin particles, reaching results similar to those of our study. The specimens of demineralized dentin showed greater exposure of the dentinal tubules and smoother surfaces compared to the non-demineralized specimens. Furthermore, the surfaces of the demineralized dentin particles demonstrated better suitability for cell proliferation (human dental pulp stem cells) than the surfaces of the non-demineralized particles. The authors also reported that the surfaces of the demineralized dentin particles were less biocompatible than those of the deproteinized dentin particles. It was suggested that these results were due to the demineralization process, during which a certain amount of the proteins may have been denatured. The authors also assumed that if the release of proteins following the demineralization process exceeds a certain threshold, it could be unfavorable, resulting in lethality for the cells.

The FT-MIR analysis was performed to investigate the functional groups on the surfaces of dentin particles [18]. This analysis offered information regarding the degree of demineralization for each specimen. The results of our study showed that the inorganic mineral component (CO_3^{2-} and PO_4^{3-}) content decreased with increasing duration of exposure to 12% EDTA. In contrast, the protein component remained unchanged. The reference spectra values were considered on the basis of findings from previous investigations [19–21].

Clearly, demineralization is effective but within a certain range. If the dentin is poorly demineralized, it results in a poorly osteoinductive scaffold. Nevertheless, if the demineralization is excessive, the substrate becomes ineffective. Therefore, an appropriate balance must be achieved. The demineralized dentine particles have a porous structure due to the presence of the dentinal tubules. This means that the dentin particles may act as an osteoconductive scaffold, which allows cells proliferation. Literature on the effects of different demineralization agent exposure times on the biological properties of dentin particles is limited. In fact, most of the studies compare specimens of demineralized dentin, non-demineralized dentin, and deproteinized dentin. To the best of our knowledge, there are no in vitro or in vivo studies regarding the effect of different durations of demineralization on dentine particles. Although the present study has some limitations, as the limited demineralized substances used and the possibility of inaccurate removal of enamel and cementum from the specimens, it can be considered a pilot study for future in vivo studies to find the best degree of demineralization that can be used as a graft material for bone regeneration.

It seems that the use of DDM offers several advantages. Both dentin and alveolar bone tissue derive from the neural crest and also share similarities in composition. Dentin consists of (i) 70% inorganic components (hydroxyapatite, tricalcium phosphate, octacalcium phosphate, and amorphous calcium phosphate); (ii) 20% organic components (mainly collagen I; collagen III and V in small quantities; non-collagenic proteins); and (iii) 10% water [10]. Non-collagenous proteins of dentin are known to be involved in bone calcification [39]. Dentin has shown not only osteoconductive but also osteoinductive properties [11,13]. Clearly, DDM can be considered an autologous graft since it is obtained from the extracted tooth of the same patient. Therefore, there is no risk of a cross-infection or rejection reaction, as confirmed by retrospective clinical studies of Lee et al. [40] and Kim et al. [41]. Although the results of in vitro and in vivo studies suggested that DDM can be considered as an alternative to autologous bone grafts [42] and xenografts [15,43], the number of clinical studies is still limited. Hence, further studies are still needed to validate the performance of DDM for clinical purposes.

5. Conclusions

Demineralized dentin matrix could be considered as a suitable alternative to autologous bone grafts and xenografts. According to previous studies, an adequate balance should be achieved in the demineralization process of dentin particles. However, due to the lack of scientific data, this study described the chemical and surface characterization of DDM after different demineralization processes. The following can be concluded:

- there is a progressive reduction in the concentration of both PO_4^{3-} and CO_3^{2-} with increasing demineralization time;
- the organic (protein) component does not change during the demineralization process;
- increasing the duration of demineralization results in dentin particles with smoother surfaces and higher numbers of dentinal tubules.

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