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ORIGINAL RESEARCH

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Comparison of clinical efficacy of three different dentin matrix biomaterials obtained from different devices

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ABSTRACT

Aim: The aim of the present study was to propose the clinical efficacy of the different dentin matrix obtained from three devices (BonMaker, Tooth Transformer, and Smart Dentin Grinder) and to show their morphological, physical, and biochemical characteristics using scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) spectroscopy, and Raman spectroscopy.
Research design and methods: The study included 70 patients who underwent bone augmentation using the BonMaker, Tooth Transformer, and Smart Dentin Grinder devices. In addition, 84 implants were placed. Furthermore, four samples, one for each device and one non-demineralized control, were analyzed with scanning electron microscopy (SEM), energy-dispersive X-ray analysis, and Raman spectroscopy.
Results: In all patients, augmentation of bone defects with ground dentin matrix was successful, and implants showed correct osseointegration. The morphological organization, the chemical composition, and the presence of organic molecules in the dentin samples processed by the three different devices were demonstrated using SEM, energy-dispersive X-ray analysis, and Raman spectroscopy.
Conclusions: Comparing BonMaker, Tooth Transformer, and Smart Dentin Grinder devices in our practice, we concluded that these systems, even with different structural and chemical differences of the dentin granules, have a comparable potential for obtaining regenerative material from the patient's own teeth.

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Demineralized dentin matrix; autogenous tooth graft; tooth grinders; scanning electron microscopy; energy-dispersive X-ray analysis; Raman spectroscopy

1. Introduction

In implant dentistry and restorative surgery, bone augmentation procedures are routinely used nowadays to treat patients with bone defects. In addition to the broadly available bone substitute materials [1], autogenous bone graft can be successfully used, as it has a specific advantage over other bone substitutes owing to its osteogenic properties [2]. However, donor graft harvesting requires an additional procedure at the donor site, which, despite the low morbidity, is associated with an increased treatment time [3]. Therefore, in the last years, the use of ground natural teeth, until recently considered as a waste material, was evaluated. From 1993, Kim et al. started experimental studies using teeth as graft material [4]. The human teeth are composed of four tissues – enamel, dentin, cementum, and dental pulp. Due to the very similar chemical composition of dentin and bone, it seems to be a very promising augmentation material. Dentin in approximately 70% is composed of inorganic hydroxyapatite crystals, in about 20% of organic extracellular matrix, mainly collagen type I and in ~10% of water [5–7]. In addition, dentin contains bone morphogenetic proteins (BMP) [8], which belong to the transforming growth factor beta (TGF-β) superfamily, which, as demonstrated for the first time by Urist [9], are able to stimulate osteogenesis. Many researchers have reported that demineralization of dentin is necessary for the release of osteoinductive growth factors trapped in dentinal tubules [10]. Several methods exist for preparation of the demineralized dentin matrix (DDM). Universally, after extraction, the patient's tooth must be cleaned of all calculus residues, soft tissues, and any foreign material. Secondly, the tooth is crushed into small particles, grinded by a special mill, and soaked in demineralizing reagents. DDM provides a scaffold to support the bone regeneration process [5]. In 2009, the first transplant with autogenous tooth graft material (AutoBT, Korea Tooth Bank Co., Seoul, Korea) was performed with no reported complications [11]. In an experimental study in 2010, the demineralized dentin matrix was obtained with a hand-operated apparatus for crushing teeth and showed the induction of bone and cartilage [12]. In a clinical study on 190 patients treated with powder-type auto-tooth bone graft material, obtained with an unspecified technique, it was concluded that it induced good bone generation through its osteoinductive and osteoconductive capacity [13]. In 2014, the results of a study on a group of 15 patients were published, demonstrating excellent healing after augmentation with autogenous teeth [14]. Based on the successful demonstration of the positive role of dentin particulate in alveolar ridge preservation

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[4,11,14], the availability of specific devices, able to produce tooth-derived material in a safe and reproducible way, was felt.

BonMaker was tested in a group of patients [15], showing, after a period of 6 months, the production of 56% new bone in treated area. In a recent case report [16], the use of partially demineralized dentin obtained from the patient's own tooth and processed with the BonMaker device demonstrated the effective regeneration of the alveolar bone defect. BonMaker presents the further advantage of producing bone blocks, which provide additional possibilities during the procedures if compared to the other devices.

Another device available on the market is Tooth Transformer. In a recent study on patient's own ground teeth processed with Tooth Transformer [17], the formation of new living bone in a regeneratively treated bone defect was demonstrated. The study also included endodontically treated teeth, which did not affect the results after augmentation procedures. In a multicentric study [18], alveolar ridge preservation was performed in 504 patients using graft material processed by Tooth Transformer, and 483 implants were placed after 4 months. After 12 months, the overall success rate was high (98.2%) and there were significantly preserved alveolar processes in all patients.

The last examined device was the Smart Dentin Grinder. At present, many authors have confirmed the clinical effectiveness of this device on both animal and human clinical trials [7,19,20]. In a group of 100 patients treated with Smart Dentin Grinder material, the repair of bone defects using patient's own ground teeth demonstrated no complications or failures over 2 years and in many subjects, implantation was performed after 2–3 months [21]. The chemical composition and morphology of Smart Dentin Grinder processed material was studied with SEM-EDX, demonstrating its significant potential for treating bone defects [22]. The efficacy of augmentation with Smart Dentin Grinder treated material was also demonstrated in a group of 15 patients, comparing the alveolar behavior after extraction of lower third molars, with augmentation on one side and with spontaneous healing on the other side [23]. It was demonstrated that filling a defect with patient's own ground tooth gave significantly better results than leaving an empty defect.

The aim of the present study was to propose the clinical efficacy of the different dentin matrix (DM) obtained from three devices (BonMaker, Tooth Transformer, and Smart Dentin Grinder) and to show their morphological, physical, and biochemical characteristics using scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) spectroscopy, and Raman spectroscopy.

2. Materials and methods

2.1. Study design

The study was approved by the Bioethical Commission of Medical University of Silesia in Katowice, Poland, No. KNW/0022/KBI/18/18 SUM of 15.05.2018 and carried out in accordance with the Declaration of Helsinki. The informed consent was obtained from all the subjects enrolled after an explanation of the nature and the possible consequences of the study. Only adult patients (over 18 years old) were considered in the study. The study was performed by two centers: Dłucik Dental Clinic, Katowice, Poland, and by researchers from the University of Messina, Messina, Italy. Design of the study is demonstrated in Figure 1.

2.2. Population

2.2.1. In vivo part

The study was conducted between September 2018 and January 2022 in Dłucik Dental Clinic, Katowice, Poland. Seventy patients were included in the study [43 females and 27 males; aged 22–77 years; mean age ± standard deviation (SD): 49.8 ± 14.3 years]. Inclusion criteria for the study were as follows: bone loss from cyst removal, two wall defects in most cases, three wall defects – patients for bone blocks, alveolar atrophy in periodontitis, extractions performed long time before, extractions of impacted teeth, and sinus lift procedures. Exclusion criteria were as follows: systemic diseases or metabolic bone disorders that could affect the healing process (e.g. uncontrolled diabetes mellitus, hyperthyroidism), cigarette smoking, chemotherapy, radiation therapy, bisphosphonates or corticosteroids treatment, pregnancy, and odontogenic acute inflammation near the surgical site. The follow-up time was 4–36 months. Our study presents the following limitations: it was not possible to control some clinical variables, such as bone graft site conditions (wall defect or amount of thread exposure) and location of bone graft (sinus graft, horizontal or vertical ridge augmentation); the aforementioned drawbacks might have introduced a bias for the objective evaluation of the clinical outcomes. Before the procedure, all patients underwent professional dental hygienization and were administered orally with clindamycin 0.6 g. In case of allergy to clindamycin, patients received amoxicillin/clavulanic acid 1 g.

The total amount of extracted teeth used as a biomaterial for bone reconstruction was 180. The extracted teeth were processed with three different devices and used in augmentation procedures (Figure 2): BonMaker (Korea Dental Solution Co. Ltd., Busan, Korea) in 38 patients, Tooth Transformer (TT Tooth Transformer Srl, Milan, Italy) in 11 patients, and Smart Dentin Grinder (KometaBio, Fort Lee, NJ, USA) in 21 patients.

Augmentations were performed in all patients using dentin matrix from ground natural teeth, either in the maxilla or in the mandible.

From the group of 70 patients, 37 had titanium implants inserted into bone augmented by DM and 33 underwent only augmentation procedures by the DM. The total number of implants, all placed with an average torque force of 35 Ncm, was 84, and in particular 51 in patients of the BonMaker group, 22 in the Tooth Transformer group, and 11 in Smart Dentin Grinder group. From the BonMaker group, 15 patients had implants placed immediately after extraction together with bone augmentation by the DDM; in addition, three block-type surgeries with immediate implant loading in esthetic area were performed in the patients from this group. In the Tooth Transformer group, four patients had implants immediately loaded into the bone alongside with augmentation by the DDM. In the Smart

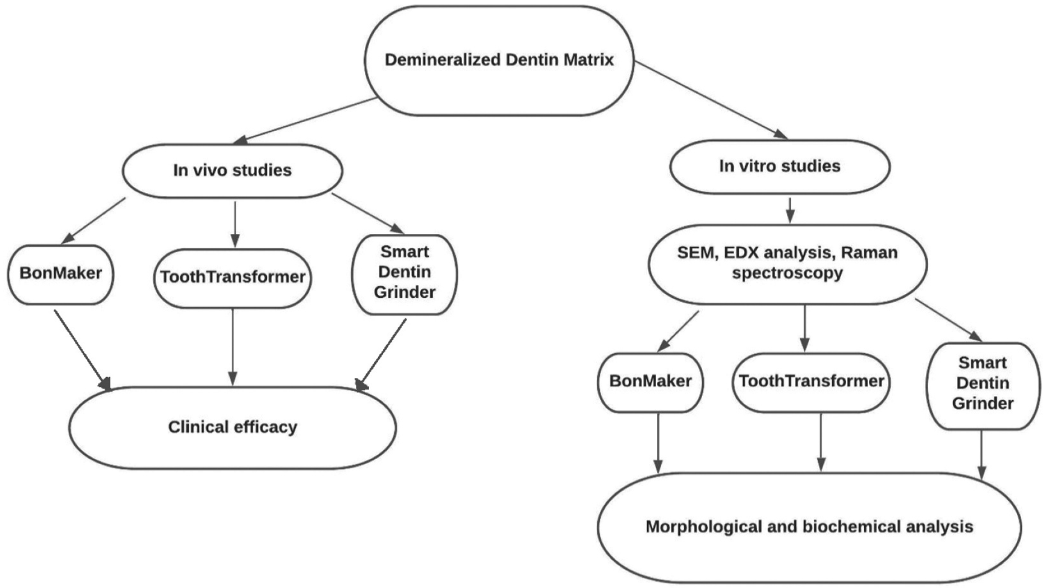

Figure 1. Flowchart presenting the study design.

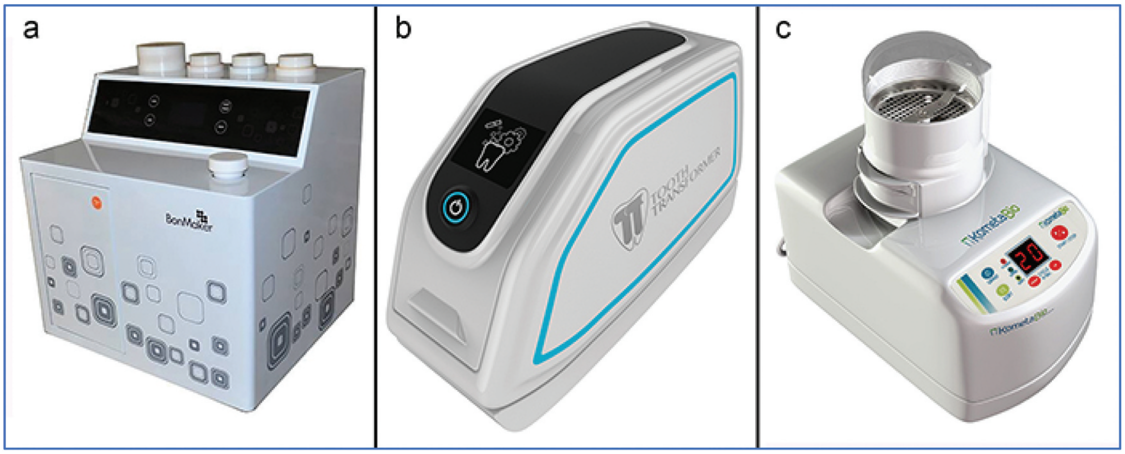

Figure 2. Different devices used to obtain dentin matrix biomaterials. a – BonMaker; b – Tooth Transformer; c – Smart Dentin Grinder.

Dentin Grinder group, three sinus lift procedures were performed, from which one patient had sinus lift with immediate implant insertion into the bone augmented by a mineralized dentin matrix (MDM). In this study, measurements were acquired via CBCT scans before tooth extractions, after surgery and 3–4 months later to evaluate the outcomes. Microbiological testing of samples coming from all three devices has been performed. Results were negative for all samples. In addition, four impacted upper third molars were extracted from four random patients (one tooth from each patient). Three samples, one for each device, were prepared, following manufacturer's instruction for sample preparation. The fourth tooth had soft tissues removed and was used as a control sample. Secondly, the tooth was placed in 3% hydrogen peroxide for preliminary disinfection, and then into a sterile saline solution in a plastic container. All four samples were sent to the University of Messina, Messina, Italy.

2.2.2. *In vitro part*

This part was conducted by the researchers from the University of Messina, Messina, Italy.

Four samples for each device and four non-demineralized controls were used for the examination with SEM, EDX analysis, and Raman spectroscopy. The non-demineralized samples were cut into two halves and used as dentin control. The SEM analysis evaluated the morphology of the granular material obtained with each device. It was coupled with EDX spectroscopy microanalysis to determine the elemental composition of the sample surface. Furthermore, Raman spectroscopy, belonging to the family of vibrational spectroscopic techniques, was also performed on the different specimens. It is a spectroscopic modality based on the inelastic scattering process of monochromatic light [24]. It is a rapid and nondestructive analysis technique for the detection of biochemical changes at the molecular level and does not require any preparation before the measurement [25]. It represents a useful methodology in various research fields, such as physics, human and veterinary medicine, chemistry, and material science [26–28]. These techniques have a large number of applications in various industrial, commercial, and research fields [29,30].

2.3. Sample preparation

Preparation of autogenous dentin grafts was performed according to the manufacturer's instructions for each device. The graft preparation for both in-vivo and in-vitro parts was performed by two surgeons from Dłucik Dental Clinic, Katowice, Poland. Selection of the device for every patient in this study was random and in decision of attending physician of the patient based on physical and radiological examination. Enamel was removed from the teeth only in case of extensive caries or large fillings.

For BonMaker [15], the extracted teeth were placed in 3% H_2O_2 for about 3′. After rinsing and drying, soft tissues and filling materials such as amalgams, inlays, and gutta-percha were removed with a slotted carbide drill. The teeth were placed in the grinder to obtain granules. Particulate material was disinfected and demineralized via specific solutions, resulting in a ready-to-use tooth graft.

For Tooth Transformer [31], the teeth were cleaned of calculus residues with a diamond drill, cut into pieces and inserted into the grinder. Particles were treated with demineralization reagent (HCL 25–50%), washed sequentially with two solutions, and treated with a sterilization reagent.

For Smart Dentin Grinder [32], we followed the mineralized protocol. The teeth were grinded to obtain a dentine granulate which was sterilized in a basic alcohol cleanser and soaked in Sodium Hydroxide and then washed with sterile saline.

2.4. Scanning electron microscopy (SEM)

Crushed particles from BonMaker, Tooth Transformer, and Smart Dentin Grinder devices were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at +4°C, washed with 0.2 M phosphate buffer (pH 7.4), dehydrated in graded ethanol, critical point dried in liquid CO_2, mounted on aluminum stubs, covered with gold, and viewed and photographed in a JEOL JCM 6000 (JEOL, Tokyo, Japan) scanning electron microscope adjusted at 15 kV.

2.5. Energy dispersive X-ray analysis and statistical analysis

One part of each control teeth and the crushed particles obtained from BonMaker, Tooth Transformer, and Smart Dentin Grinder devices, all treated as above indicated for SEM, were air-dried, mounted on aluminum stubs, and examined for elemental analysis by the energy-dispersive X-Ray analyzer (EDX) Jeol DX200s (JEOL, Tokyo, Japan). An X-ray spectrum was obtained, and the elements from each specimen were identified from the peak energy and quantitatively analyzed from the intensity of the peaks. The results were obtained by referring to the energy spectra of calibration included in the instrument library. The qualitative and quantitative elemental analyses were performed at different points of the surface of each sample, and the obtained results were related to the mean value of the measurements.

Statistical analysis was performed using MATLAB (version R2018b; MathWorks Inc., Natick, MA). Different measure groups were compared using the z-test, which allows us to determine whether two groups of measurements follow the same distribution. The z-statistics were calculated using the formula:

$$Z_{ij} = \frac{\bar{x}_i - \bar{x}_j}{\sqrt{\sigma_i{}^2 + \sigma_j{}^2}},$$

where $\bar{x}_i$ and $\bar{x}_j$ are the mean values of the i-th and j-th datasets, and σ_i and σ_j their standard errors. The corresponding p-value was calculated using the cumulative distribution function (CDF, MATLAB function 'cdf') of the standard normal distribution. $P \le 0.05$ was considered statistically significant. Comparisons were not performed in groups where no measurement value could be obtained due to too low elemental concentration.

2.6. FT Raman spectroscopy analysis

Raman measurements were made from the air-dried second part of the control teeth and from BonMaker, Tooth Transformer, and Smart Dentin Grinder dentin crushed particles with a DXR-SmartRaman Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) using a diode laser with the excitation wavelength of 785 nm. The particles, in powder form, were examined using a pellet holder. This device contains a removable metal disk, in which the powdered samples could be compressed to create a pellet. Powdered samples were accommodated into the pellet holder, and the 180 Degree Sampling Accessory was used for measurements. In order to obtain high signal-to-noise ratio (S/R) spectra, each Raman spectrum was obtained from 32 sample exposures; the length of each exposure during data collection was set to 60.0 s. Total acquisition time was, therefore, 32 minutes for each spectrum. All the Raman spectra were stored in .SPA format and the post-processing analysis was performed using the Omnic for dispersive Raman 9.0 software. After collection, the band centered at about 957 cm^{-1} was used as an internal standard to normalize the spectra.

The degree of sample mineralization (DM) was calculated by using the following formula:

$$DM(\%) = \left(1 - \frac{I_{2900}}{I_{957}}\right) x100$$

where I_{957} is the peak intensity of the band centered at 957 cm^{-1} and I_{2900} is the intensity of the band located around 2900 cm^{-1}.

3. Results

3.1. Clinical data

The collected data are presented in Table 1 (BonMaker), Table 2 (Smart Dentin Grinder), and Table 3 (Tooth Transformer). Tooth numbers were expressed according to the recognized two-digit FDI Dental Numbering System. In all 70 patients, the alveolar processes were successfully regenerated, and all 84 implants osseointegrated properly. In all cases studied, there were no

Table 1. Comparison of different methods for dentin matrix preparation.

Method of DM preparation	Size of granules	Main reagent	DM processing time	Automation of the procedure
BonMaker	From 500 to 1000 µm	hydrochloric acid (HCL) with a concentration between 3, 5-5%	~20 minutes (ATB block ~35 minutes, 50 seconds)	Semi-automated
Tooth Transformer	From very small (20–40 µm) to larger (1.0–1.2 mm)	Hydrochloric acid (HCL) with a concentration between 25% and 50%	~30 minutes	Fully automated
Smart Dentin Grinder	From 300 µm to 1.3 mm	Sodium hydroxide, 0.5 M solution	~15–20 minutes	Semi-automated

Microbiological testing of samples coming from all three devices has been performed. Results were negative for all samples.

significant complications during the recovery period that could affect the assessment of patient's healing.

On the basis of radiological (CBCT) examination, in most cases, implantation could be performed after a period of ~3 months. In the follow-up period, we did not observe any marginal bone loss around implants that were inserted into bone augmented by DDM and DM. In the case of patients who underwent only augmentation procedures, no significant bone resorption was observed during follow-up, regardless of the method used. The vertical and horizontal dimensions of the alveolar ridge were preserved or even increased after 3–4 months from bone regeneration and remained stable after at least 6 months from the implant placement. Prosthodontic part could be started in most cases after 3 months in the mandible and around 4 months in the maxilla.

The present study confirmed the effectiveness of all three devices in bone augmentation procedures and implantations, as no failures were reported.

3.2. Scanning electron microscopy

When the specimens obtained from BonMaker were observed with SEM, at low magnification (Figure 3a) the particles showed a rather uniform size, varying from 0.5 to 1.0 mm. At higher magnification (Figure 3b), some particles showed irregular margins, on which the orifices of the dentinal tubules were evident. Other particles showed margins in the form of staircase steps (Figure 3c). At higher magnification (Figure 3d), dentinal orifices and/or tubules were present, according to the plane of grinding.

In the specimens obtained from Tooth Transformer, SEM observation demonstrated, at low magnification (Figure 4a), particles with different sizes, varying from very small fragments of about 20–40 µm to larger samples of 1.0–1.2 mm. All the particles showed smooth surface (Figure 4b); at higher magnification, the orifices of dentinal tubules were evident (Figure 4c). Round rims of dentinal tubules and longitudinal tubules were present, according to the plane of grinding (Figure 4d).

In the specimens obtained with Smart Dentin Grinder, SEM observation demonstrated, at low magnification (Figure 5a), the particles had sharp margins and sizes varying from 300 µm to 1.3 mm. The surface of each particle was generally flat, with occasional linear reliefs (Figure 5b), which, at higher magnification (Figure 5c), had a lamellar shape with dentinal tubules and orifices. Other particles showed deeper and regular furrows, particularly on their lateral surfaces (Figure 5d).

3.3. Energy dispersive X-ray analysis

The EDX analysis was applied to detect the elemental composition of BonMaker, Tooth Transformer, and Smart Dentin Grinder crushed particles when compared to the control dentin. The obtained results are reported in Figure 5. The Al (Kα) contribution was not considered as it was related to the specimen stubs.

In dentin specimen (Figure 6a) C, O, P, and Ca were the major components; the high concentration of Ca and P reflected the mineral content in the sample. On the contrary, in BonMaker specimens (Figure 6b), the major elements sampled were C and O, with only small traces of N, Mg, and Si, thus indicating a deep demineralization of the sample examined; in fact, Ca was not detected and only traces of P were found. Similarly, Tooth Transformer samples showed a demineralization not as effective as in the BonMaker (Figure 6c); the presence of Ca (K_α) was about a half if compared with the dentin samples, while P and Ca (K K_α) were comparable in percentage with dentin sample. In Smart Dentin Grinder specimens (Figure 6d), the highest element sampled was Ca, whose percentage was higher than 50%. C, O, and P were also detected, with percentages closer to dentin element values.

Statistical analysis (Figure 7) revealed that C concentration was significantly different ($P < 0.05$) for all comparisons, except Tooth Transformer versus Smart Dentin Grinder ($P = 0.02$). For N, statistically significant differences were observed only for BonMaker versus the other groups, while for O and Na only Tooth Transformer values were significantly different versus the other groups. No significant differences among the groups were found for Mg, Si, and K ($P > 0.05$). As to P and Ca(Kβ), the values of dentin, Tooth Transformer, and Smart Dentin Grinder were statistically significant only versus BonMaker. Lastly, for Ca(Kα), significant differences were observed among all groups.

3.4. FT Raman spectroscopy analysis

The mean spectra of control dentin (Figure 8a) and of BonMaker (Figure 8b), Tooth Transformer (Figure 8c), and Smart Dentin Grinder (Figure 7d) particles were determined by using the OMNIC for dispersive Raman 9.0 software and the resulting spectra were observed over the wave number range of 3200 and 200 cm^{-1}. The wave number ranges and their relative assignment, according to the literature, are reported in Table 4.

The most intense band was the strong region centered at 957 cm^{-1}, due to the inorganic component, corresponding to

Table 2. Clinical data (age, sex, diagnosis, implant site, source of DDM, and implant diameter and length) of patients processed with BonMaker. IA = implant augmentation; DDM = demineralized dentin matrix.

No. Patient	Diagnosis	Implant augmentation site	Source of DDM	Implant diameter [mm]	Implant length [mm]	IT [Ncm]	Legend
1	21 – Vertical root fracture	21	21 radix relicta, 38 – impacted tooth	3,8	13	35	DDM = demineralized dentin matrix, IT = insertion torque)
2	Crown fracture	24	24	3,8	11,5	35	
3	Post-traumatic osteolysis around root 11	11	11 root, 28 – vestibular eruption	4,5	13	35	
4	Crown fracture	26	Roots of 26	4,5	8,5	35	
5	Pulp necrosis	16	16	4,5	10	35	
6	Root fracture	15	15	4,5	7	35	
7	Osteolytic changes	16	16	5	11,5	35	
8	Periapical changes	11,21	11,21,28	11:3,8;21:3,8	11:13;21:13	35	
9	Periapical changes	11,21,23,25	11,21,23,25,27	11–4,5;21–4,5;23–4,5;25–3,8	11–11,5;21–11,5;23–11,5;25–10	35	
10	Periodontal disease	13–21	13,12,11	13:4,5;12:4,0;21:4,0	13:11,5;12:13;21:13	35	
11	Periodontal disease	16, 14,21,22,23,25,26	14,21,22,23,25,26	16–5,0; 14–4,5;21–3,8;22–3,8;23–4,0;25–4,5;26–4,5	16–13;14-13;21–11,5;22–13;23–13;25–13;26-8	35	
12	Periodontal disease	16,17, 21,23,25	Whole 17, 16,21,27	23–3,8; 25–4,0	23–11,5; 25–10	35	
13	Pulp necrosis	23,25	17	23–3,3;25–3,3	23-13;25-10	35	
14	Periodontal disease	25,26	25,26	25:4,5;26:5,0	25:11,5;26:8,5	35	
15	Periapical changes	43, 46,47	36,37	4,3–3,8;46–4,0;47–4,0	43–13;46–10;47–10	35	
16	Periodontal disease	43, 45,32,34,37	34,35,37,43	43–4,0;45–4,5;32–4,0;34–5,0;3,7–5,0	43–11,5;4,5–8,5;32–11,5;34–11,5;37–8,5	35	
17	Periimplantitis	44, 47	18 – vestibular eruption, 35-radix relicta	44–3,8 47–4,5	44 – 8,5; 47–7,5	35	
18	Horizontal and vertical bone loss after extraction	46–36	Impacted tooth 48, radix relicta 46	46,36–4,5	46,36–13	35	
19	Periodontal disease	46,44,41,31,34,36	45,44,43,41,42,31,32,33,34,35	46–4,0;44–4,5;41–4,0;31–4,5,34–4,5;36–4,0	46-10; 44–13;41-13;31–11;34-11,5;36–10	35	
20	Periodontal disease	45,37	45,37	45:4,5;37:4,0	45:4,5;37:8,5	35	
21	Cyst around impacted tooth	38	Whole lower third molar				
22	Impacted upper wisdom tooth	28	Whole upper third molar				
23	Periodontal disease	17,15,14,13,22,25,27	17,15,14,13,22,25,27				
24	Horizontal and vertical bone loss after extraction	36	28 impacted upper wisdom tooth				
25	Periapical cyst	35,36	35,36				
26	Periapical changes	15,27,47	15,27,47				
27	Periodontal disease	14,25,27	14,25,27				
28	Periimplantitis	46	38				
29	Palatal impaction 15	15	15				
30	Large radicular cyst	14,11	14,13,12,11				
31	Periapical changes	16	17				
32	Radicular cyst	14–11	16,14,12,11,21,24,25,27				
33	Periodontal disease	47–44	48,46				
34	Pulp necrosis and radices relicta	14,15,24	16, 14,24				
35	Large radicular cyst	13–22	16,13,21				
36	Palatal impaction	25	25				
37	Large radicular cyst	16	18,16				
38	Periodontal disease	11,21	11,21				

Table 3. Clinical data (age, sex, diagnosis, implant site, source of DM, and implant diameter and length) of patients processed with Smart Dentin Grinder. IA = implant augmentation; DM = dentin matrix.

No. Patient	Diagnosis	Implant augmentation site	Source of DM	Implant diameter [mm]	Implant length [mm]	IT [Ncm]	Legend
1	Radicular cyst	35	35	4,50	11,50	35,00	DDM = demineralized dentin matrix, IT = insertion torque)
2	Radicular cyst	16	16	5,00	7,00	35,00	
3	Periodontal disease	17,16,25,26	16,17,27	17:5,0:16:5,0;25:3,8;26:3,8	17:10;16:11,5;25:11,5;26:11,5	35,00	
4	Impacted upper canine	13	13, 53	3,80	13,00	35,00	
5	Periodontal disease and sinus lift	17	47,37	5,00	10,00	35,00	
6	Crown fracture	21	21,38	3,80	13,00	35,00	
7	Periapical changes	14–15	18,16	14:4,0;15:4,5	14:8,5;15:7	35,00	
8	Impacted lower third molar	38	38				
9	Radicular cyst	16	16				
10	Periodontal disease and sinus lift	24–26	42,41,31,32				
11	Radix relicta	23–26	23,26				
12	Radicular cyst	12	18,17,16,12,47				
13	Radix relicta	14	14				
14	Radix relicta	25	38,25				
15	Periapical changes	36,37	18,36,37				
16	Periapical changes	14	18,14				
17	Radicular cyst	12	12,22,24				
18	Pulp necrosis	16	16				
19	Impacted lower canine	43	43				
20	Periodontal disease and sinus lift	24–27	41,42,47,24				
21	Radicular cyst and radix relicta	11,21	11,21, mesiodens				

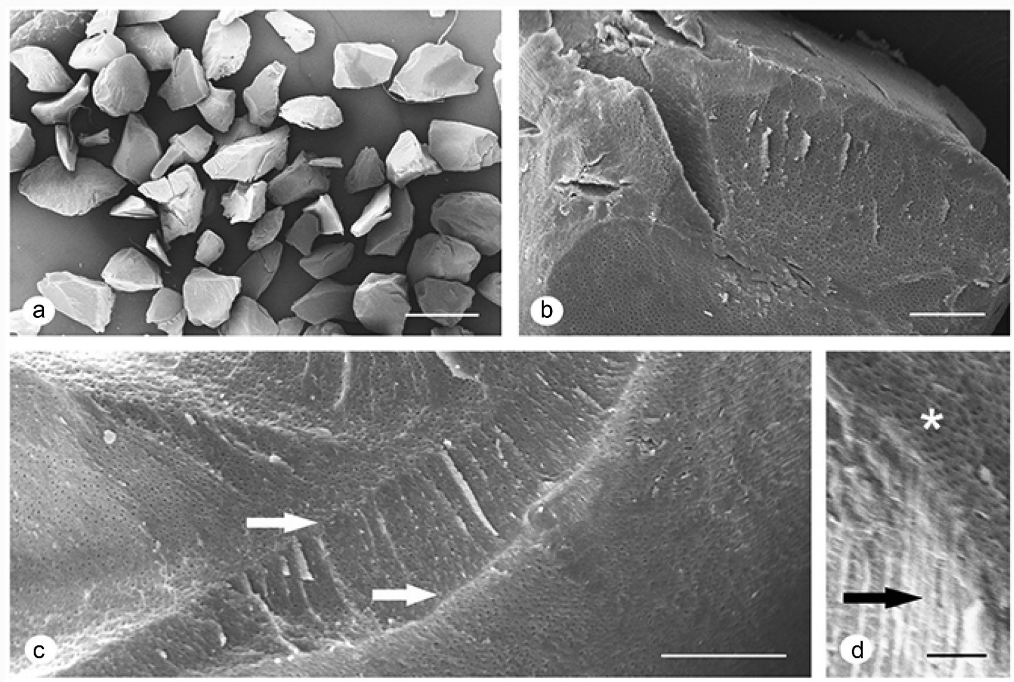

Figure 3. Scanning electron microscope micrographs of BonMaker crushed particles. a: At low magnification, the particles show a rather uniform size, varying from 0.5 to 1 mm. b: At higher magnification, some particles show irregular margins on which the orifices of dentinal tubules are evident. c: Other samples show margins arranged as staircase steps (arrow). d: At higher magnification orifices (arrow) and dentinal tubules (*) can be demonstrated. (Scale bar: a = 1 mm; b = 200 µm; c = 100 µm; d = 20 µm).

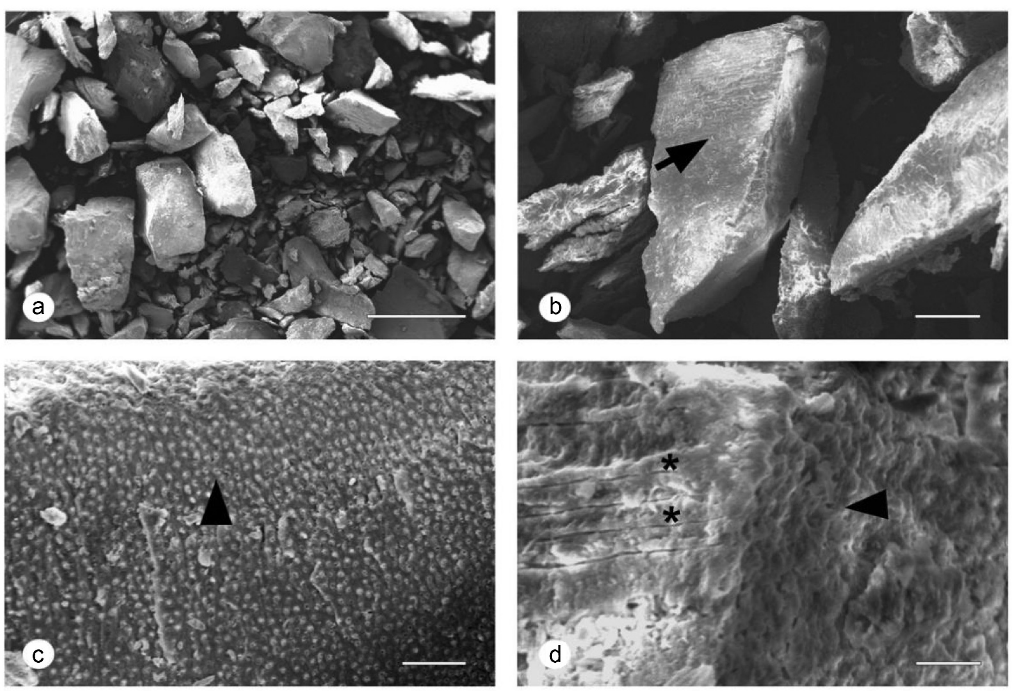

Figure 4. Scanning electron microscope micrographs of Tooth Transformer crushed particles. a: At low magnification, the particles show different size, varying from very small fragments of about 20–40 µm to larger samples of 1.0–1.2 mm. b: All particles show smooth surface (arrow). c: At higher magnification, dentinal tubules can be demonstrated on the surface (arrowhead). d: Round rims of dentinal tubules (arrowhead) and longitudinal tubules (asterisk) are present. (Scale bar: a = 1 mm; b = 200 µm; c = 40 µm; d = 10 µm).

the v_1 $(PO_4)^{3-}$ phosphate, which was used to normalize all the spectra. The dominant v_1 $(PO_4)^{3-}$ phosphate transition arises from the totally symmetric (non-degenerated) P-O bond stretches. Other vibration modes of phosphate associated with the inorganic mineral component are the v_2 $(PO_4)^{3-}$ phosphate transition due to doubly degenerate O-P-O bending modes and the v_4 $(PO_4)^{3-}$ phosphate transition due to triply degenerate O-P-O bending modes.

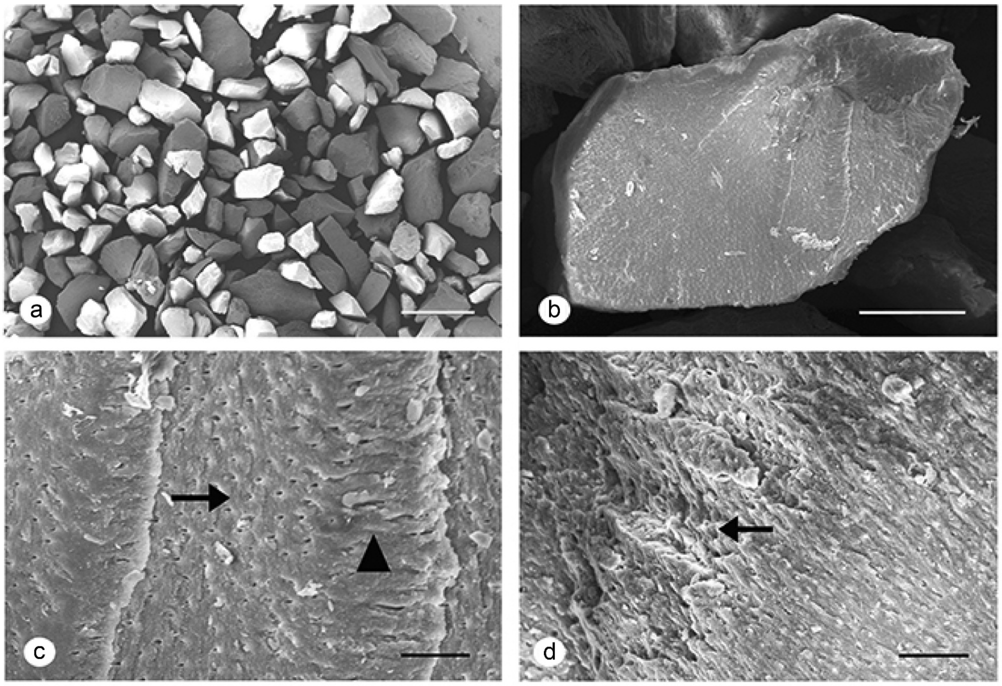

Figure 5. Scanning electron microscope micrographs of Smart Dentin Grinder crushed particles. a: At low magnification, the particles have sharp margins and size varying from 300 µm to 1.3 mm. b: The surface of each particle is generally flat, with occasional linear reliefs. c: At higher magnification, the reliefs have a lamellar shape and showed both the dentinal longitudinal tubules (arrowhead) and orifices (arrow). d: Some particles show deeper and regular furrows (arrow), particularly on their lateral surfaces. (Scale bar: a = 1 mm; b = 200 µm; c-d = 20 µm).

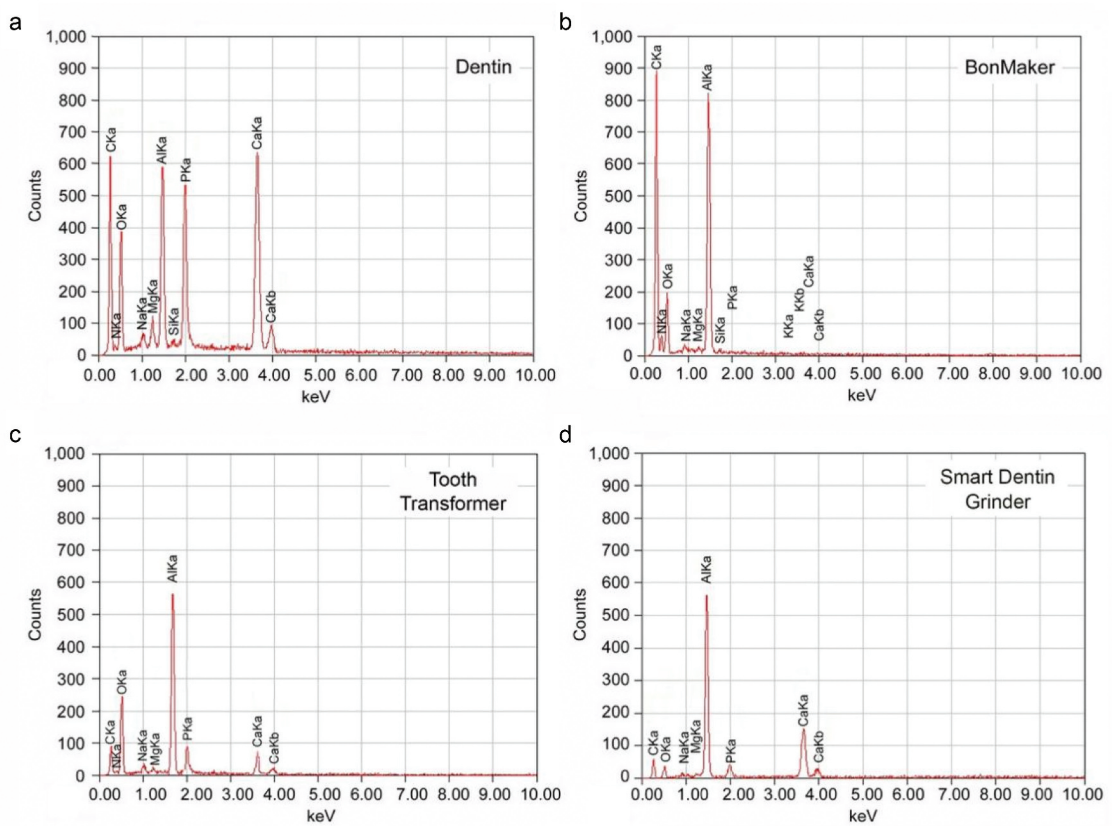

Figure 6. Results of energy-dispersive X-Ray analysis. a: Elemental analysis of dentin. b: Elemental analysis of BonMaker crushed particles. c: Elemental analysis of Tooth Transformer crushed particles. d: Elemental analysis of Smart Dentin Grinder crushed particles. The Al (Kα) contribution was not considered because it was related to the specimen holder.

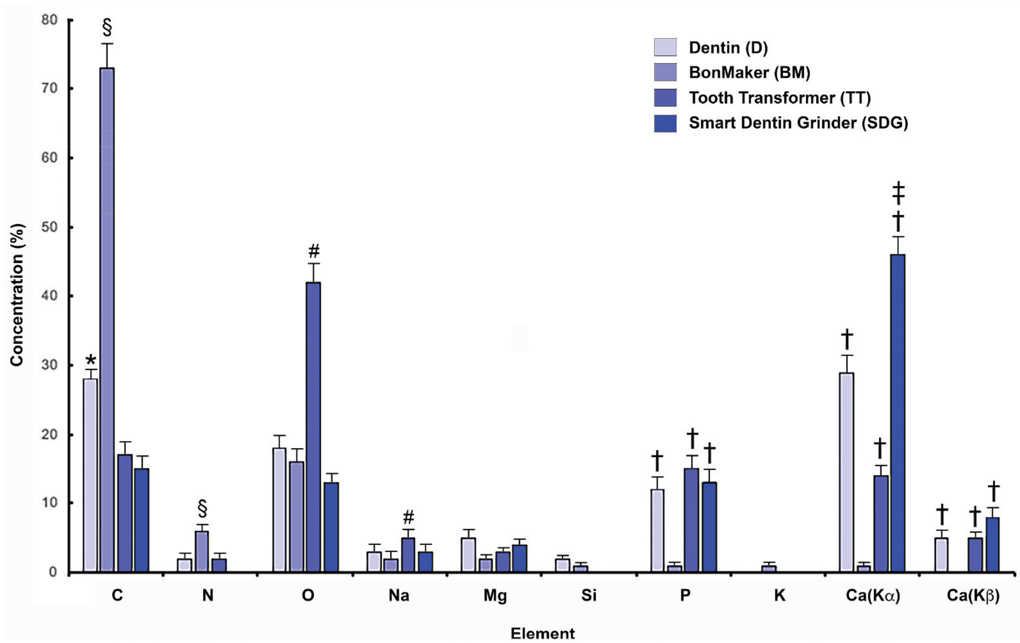

Figure 7. Percentage concentration and statistical evaluation of the detected elements in the samples examined with EDX-SEM analysis. * $P < 0.05$ vs BM, TT, SDG; § $P < 0.05$ vs D, TT, SDG; # $P < 0.05$ vs D, BM, SDG; † $P < 0.05$ vs BM; ‡ $P < 0.05$ vs D, TT.

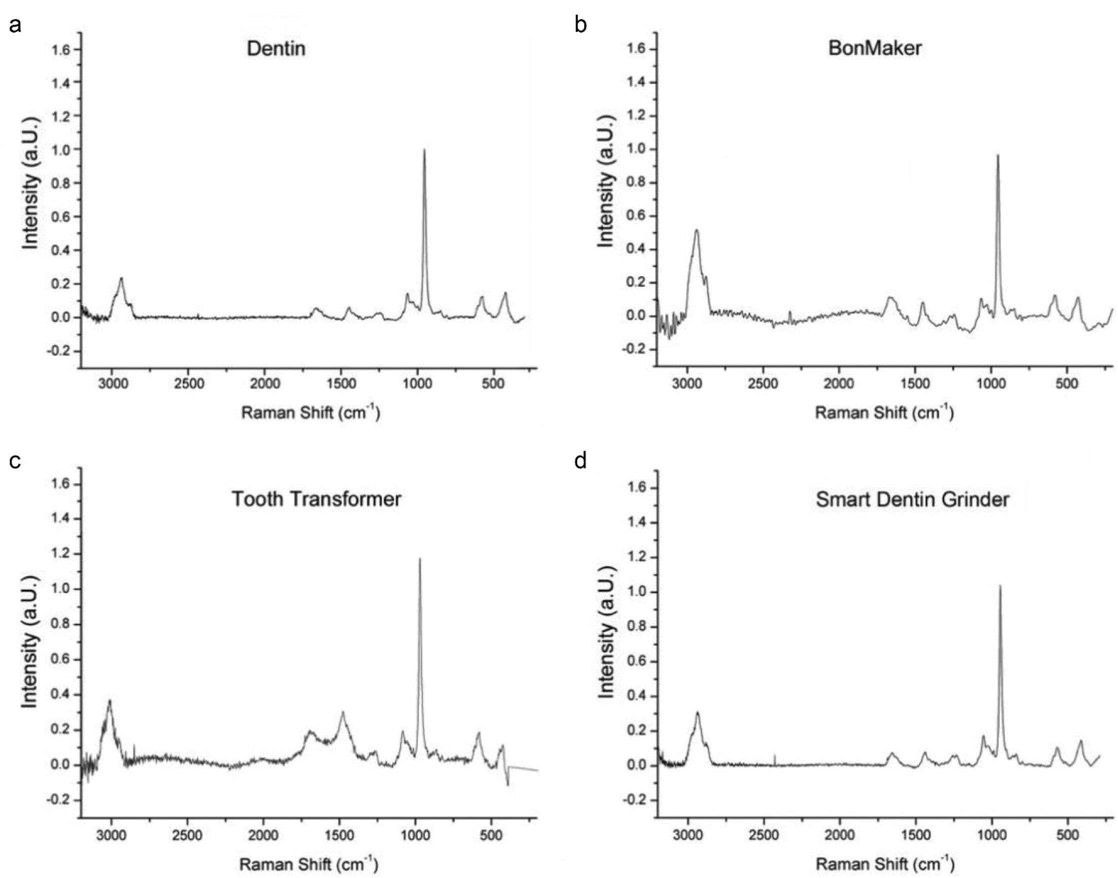

Figure 8. Raman spectra of control dentin (a), and of BonMaker (b), Tooth Transformer (c), and Smart Dentin Grinder (d) crushed particles over the wave number range of 3200–200 cm^{-1}. All the Raman intensity values are normalized to the intensity value of the ν 1 $(PO_4)^{3-}$ band centered at 957 cm^{-1}.

Table 4. Clinical data (age, sex, diagnosis, implant site, source of DDM, and implant diameter and length, insertion torque force) of patients processed with Tooth Transformer. IA = implant augmentation; DDM = demineralized dentin matrix.

No. Patient	Diagnosis	Implant augmentation site	Source of DDM	Implant diameter [mm]	Implant length [mm]	IT [Ncm]	Legend
1	Radix relicta	12	12	4,00	13,00	35,00	DDM = demineralized dentin matrix, IT = insertion torque)
2	Root fracture	12	18 – vestibular eruption, 48 – impacted	4,50	14,00	35,00	
3	Pulp necrosis	16	16 – Pulp necrosis, 28 – vestibular eruption	4,50	8,50	35,00	
4	Periapical changes	12, 14	12,14	12–3,8;14–3,8	12–11,5;14–11,5	35,00	
5	Periodontal disease	13,12,11,21,22,23,28	13,12,11,21,22,23,28	13:3,8;11:3,8;21:4,0;23:3,8	13:11,5;11:13;21:12,5;23:11,5	35,00	
6	Periodontal disease	44,41,31,34	44,41,31,34	41,:3,8;31:3,8;44:3,8,34:3,8	41:13;31:13;44:11,5;34:11,5	35,00	
7	Crown fracture and periodontal disease	23,25	23 – crown fracture, 24,37 periodontal changes	25:4,5;23:4,0	25:8,5;23:10	35,00	
8	Peridontal disease	16,17	16,17	16:4,5;17:5,0	16:8,5;17:8,5	35,00	
9	Periapical changes	23,25	17	23:3,3;25:3,3	23:13;25:10	35,00	
10	Periodontal disease	17–23	17,16,14,11,21,22,23,				
11	Radicular cyst	14	18,14				

Table 5. Raman spectroscopic bands assignments for dentin, BonMaker, tooth transformer, and smart dentin grinder specimens.

Wave number range (cm^{-1})	Assignment	Reference
400–474	$(PO_4)^{3-}$ ν$_2$ phosphate	[33]
555–625	$(PO_4)^{3-}$ ν$_4$ phosphate	[34]
942–970	$(PO_4)^{3-}$ ν$_1$ phosphate	[33]
1049–1083	$(CO_3)^{2-}$ ν$_1$ carbonate	[35]
1213–1298	Amide III	[36]
1432–1468	CH_2 wag	[34,35]
1583–1717	Amide I (C=O)	[33,34]
2843–3005	CH_2 stretching	[33–36]

Another strong Raman signal was detected in the wave number region of 2843–3005 cm^{-1}. This region was assigned to the CH_2 stretching vibration, characteristic of the organic component, together with peaks due to the organic component of dentine, that were related to vibration of the groups NH of the amide III band (wave number range 1213–1298 cm^{-1}), CH_2 wag (wave number range 1432–1468 cm^{-1}) and C=O stretching of the amide I band (wave number range 1583–1717 cm^{-1}).

In Table 5 we reported the degree of mineralization percentage calculated using the equation described in Materials and Methods section for each analyzed sample by Raman spectrometer, which confirmed the composition of the mineral phase of the dentin [37], when compared to the different samples obtained with the studied devices.

4. Discussion

A new era in alveolar bone regenerative treatment started when Kim et al. [11,14] developed a novel method for treating bone defects using autogenous dentin. Their results encouraged other scientists, to develop devices for preparation of sterile bone graft material derived from patient's own ground teeth, which is a promising alternative to traditional autogenous transplantation, due to the ease and speed of preparation, and to the absence of additional procedures to harvest bone.

When the ultrastructural features of the particles obtained with the different devices were considered, SEM showed that they differed in shape and size. In fact, in the samples from BonMaker, the largest number of particles showed a geometric shape and a rather uniform size, ranging from 500 to 1000 µm. In the samples obtained from Tooth Transformer, the particles were prismatic or lamellar and their size varied from very small (20–40 µm) to larger (1.0–1.2 mm). In Smart Dentin Grinder samples, the particles had a geometric shape and ranged in size from 300 to 1300 µm. The suitable form and size of dentin matrix to be used as a bone substitute were the argument of several studies [38–41]. In fact, while a superior role in bone formation was described with particles of bovine bone about 300 µm in size in highly mineralized tissues [38,40], an optimal bone regeneration from dentin matrix was observed with larger particles around 1000 µm in size obtained from rabbit bone [39] or from human teeth [41]. Therefore, the size of the samples obtained from the three different devices evaluated in our study can be considered adequate for their use as graft material for bone regeneration. SEM exam of the particles obtained with the different devices showed at higher-magnification, well-evident dentinal tubules. These structures move centrifugally from the pulp to the enamel-dentin border and contain odontoblast processes and interstitial fluids to form and maintain the dentine; around each process, the dentine matrix mineralizes, thus forming a dentinal tubule [42]. The presence of well-preserved dentinal tubules in all the examined specimens was an important feature, as it was demonstrated that when the demineralized dentin material is in contact with a bone defect area, mesenchymal cells differentiate into osteoblasts which secrete the matrix, soon mineralized, and then into osteocytes. The processes of osteocytes form a network on the dentinal surface, some of them extend into the dentinal tubules and combine with each other: the degree of dentinal invasion was demonstrated of approximately 5 µm from the interface [43]. Under this aspect, all samples from the examined devices showed morphological features indicating the presence of a structural basis for an adequate bond between dentin and bone.

Another aspect worthy of evaluation is the importance of the demineralization level of the dentin matrix, which is also related to the time and to the reagents used, which vary in the procedures of each examined device as reported in Table 6. The EDX analysis and the percentage of mineralization obtained by Raman Spectrometer showed that the material obtained with Smart Dentin Grinder exhibited the highest similarity in elemental concentration with dentin among the three tested devices, in particular when the concentration of Ca(Kα) is considered. On the contrary, Tooth Transformer and BonMaker exhibited significant percentage differences versus dentine. According to previous findings [42], the level of dentin matrix demineralization affects the formation of new bone. In fact, it was reported that partially demineralized dentin matrix (PDDM) provides better results than MDM [10], as dentinal growth factors, trapped in non-demineralized dentin matrix, are released after demineralization [43]. Among them, transforming growth factor (TGF)-β1, bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF), and insulin-like growth factor-1 (IGF-1) play a significant role in stimulating tissue regeneration [44]. Another study revealed that PDDM gave better results than completely demineralized dentin matrix (CDDM) [41], as growth factors were not inactivated during the process [8].

When the clinical data are compared with the results of SEM-EDX, it was possible to propose that even absolute quantity of Ca mildly lower than normal dentin is able to induce bone regeneration. According to the manufacturer, in the case of Smart Dentin Grinder, we followed the mineralized protocol; however, in our opinion, it still results in partial demineralization (in lower extent than in the other two devices) by Sodium Hydroxide, as dentinal tubules are visibly open in Figure 4. The results of elemental analysis in Figure 6 and

Table 6. The degree of mineralization percentage [DM (%)] obtained with the equation described in Materials and Methods section is reported for each analyzed sample by Raman spectrometer.

Sample	DM (%)
Dentine	72 ± 4
BonMaker	45 ± 2.7
Tooth Transformer	60 ± 3.8
Smart Dentin Grinder	69 ± 4.2

the degree of demineralization shown in Table 6 pushes us to such considerations. Raman spectroscopy, in addition to the presence of crystalline phosphate-based minerals [45], confirmed that many organic molecules were present in all the samples obtained by the different devices. In fact, the groups NH of the amide III band (wave number range 1213–1298 cm^{-1}), CH2 wag (wave number range 1432–1468 cm^{-1}), C=O stretching of the amide I band (wave number range 1583–1717 cm^{-1}) and the CH2 stretching (wave number range 2843–3005 cm^{-1}) can be considered characteristic of the organic part of dentin [46,47]. In particular, as type I collagen accounts for 90% of the dentin protein fraction [4], providing a scaffold for the minerals together with phosphophoryn [48], its presence in all examined specimens indicated the maintenance of a well-evident organic material, despite different procedures and the time of exposure of the teeth to different demineralizing reagents. However, in terms of composition of organic matter, BonMaker and Tooth Transformer samples showed a higher intensity in spectroscope when compared to Smart Dentin Grinder as shown in Figure 8.

A satisfactory end result was obtained regardless of the procedure used. Augmentations using patient's own ground teeth do not require the use of bio collagen membranes [49], thus significantly reducing the cost of the procedure. Not without significance is the place of bone regeneration. In lateral parts of the maxilla and mandible, all three devices are comparably effective. However, in esthetic region, the lack of labial bony wall of a maxillary anterior part of alveolar process, especially three wall defects, makes BonMaker the best device in this case, because of the possibility to produce 'bone' block from the tooth. The use of bone block results in a much better esthetic effect in the final prosthetic restoration of the implant in this area, as we have demonstrated in our previously published article [16]. Another aspect worth discussing is the automation of DM preparation in case of each device. Tooth Transformer is fully automated, while BonMaker and Smart Dentin Grinder are semi-automated devices. The semi-automation of the procedure results in increased time spent by the surgeon in the process of preparation of DM as shown in Table 1.

When analyzing the available literature on ground dentin matrix in the rehabilitation of bone defects, the most relevant reports were selected by referring to the devices used to prepare it.

Regardless of material and methodology, all authors have had clinical success in their studies up to date which is encouraging.

5. Conclusions

Within the limitations of the present study and on the basis of our clinical observations, all three devices are very useful in bone regenerative treatment. The size and shape of the particles obtained with our SEM-EDX studies did not influence the clinical results. Studies using Raman spectroscopy showed that BonMaker and Tooth Transformer, with a higher degree of demineralization, showed a much greater presence of organic molecules in the tested samples from Smart Dentin Grinder, which also had no noticeable influence on the final therapeutic effect.

Most of our patients could have an implant inserted after a period of about 3 months from bone grafting, regardless of the procedure used. Further studies must be performed to evaluate the efficacy of the dentin matrix. In our practice, all three devices will be used for bone regeneration.

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Data Availability Statement

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethical approval

The study was approved by the Bioethical Commission of Medical University of Silesia in Katowice, Poland, No. KNW/0022/KBI/18/18 SUM of 15.05.2018 and carried out in accordance with the Declaration of Helsinki. The informed consent was obtained from all the subjects enrolled after an explanation of the nature and the possible consequences of the study. Only adult patients (over 18 years old) were considered in the study. The study was performed by two centers: Dłucik Dental Clinic, Katowice, Poland, and by researchers from the University of Messina, Messina, Italy.

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