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ORIGINAL RESEARCH

Histological examination of tooth-derived biomaterials obtained from different devices

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ABSTRACT

Aim: The aim of the present study was to investigate the histological differences between samples processed by different devices and to confirm safe clinical application of different dentin matrix obtained from three devices: BonMaker, Tooth Transformer, and Smart Dentin Grinder in regeneration of maxillary defects.

Research design: The study involved 39 patients with two-wall or three-wall defects who underwent bone augmentation procedures in the maxilla using dentin matrix grafts from the BonMaker, Tooth Transformer, and Smart Dentin Grinder devices. Histological examination was conducted on samples obtained from patients who received each device. In this article, histological samples have been selected and are presented.

Results: In all patients, bone defects were successfully augmented with ground dentin matrix. The histological examination revealed no inflammation and a good connection between the bone and dentin matrix and clinically all patients were qualified for implant placement.

Conclusions: After comparing the BonMaker, Tooth Transformer, and Smart Dentin Grinder devices in our practice, we concluded that all these systems have the potential for obtaining regenerative material from the patient's teeth.

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KEYWORDS
Demineralized dentin matrix; autogenous tooth graft; tooth grinders; bone augmentation procedures; histological examination

1. Introduction

The loss of teeth often leads to vertical and horizontal atrophy of the alveolar process, which necessitates the use of augmentation procedures for successful implant placement [1]. While a wide range of bone substitute materials is available, the gold standard in implantology remains autogenous grafts due to their properties [2,3]. However, the availability of autogenous material is limited and the need for additional intra-oral or extra-oral procedures increases the risk of complications and prolonged healing time, which may not be feasible for all patients [4]. Ground natural teeth, classified as autogenous grafts, have recently demonstrated effectiveness in the treatment of bone defects [5,6]. Researchers around the world have patented devices for converting dentin matrix (DM) into sterile augmentation material, which dentists are successfully using with promising research results [7–9]. The use of previously disposed natural teeth has become possible due to the similar structure of dentin to the bone. Dentin, physiologically and anatomically, is a complex structure composed of approximately 70% inorganic compounds, mainly hydroxyapatite crystals, around 20% organic compounds, mainly type 1 collagen, and approximately 10% water [10]. Bone grafts serve to provide mechanical support and stimulate osteogenesis. The decalcification process of dentin matrix preparation devices leads to the opening of dentinal tubules, which subsequently results in the gradual release of growth factors, including bone morphogenetic proteins (BMPs), that promote osteogenesis [11,12]. This study (Figure 1) aimed to evaluate healing and safe clinical application based on histological findings in three groups of patients who underwent augmentation procedures using dentin matrix processed by three different devices – BonMaker (BM), Tooth Transformer (TT), and Smart Dentin Grinder (SDG). In this article, samples processed by BM and TT results in demineralized dentin matrix (DDM) while SDG samples results in mineralized dentin matrix (MDM).

2. Materials and methods

The study was approved by the Bioethical Commission of the Medical University of Silesia in Katowice, Poland, under the number KNW/0022/KBI/18/18 SUM and was carried out under the Declaration of Helsinki.

2.1. Population

2.1.1. In vivo studies

This retrospective study was conducted between May 2020 and November 2022 by two centers: the clinical part was carried out at Dłucik Dental Clinic (DDC), Katowice, Poland, while the histological part was performed at the Department of Histology and Embryology, Medical University in Katowice, Poland. Only adult patients (over 18 years old) were considered for the study.

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A total of 39 patients were included in the study, of which 25 were female and 14 were male, with an age range of 18–77 years (mean age ± standard deviation: 50.589 ± 14.862 years). All individuals received a thorough explanation of the study and signed their written informed consent before being included. The potential risks, possible complications, and benefits of the proposed treatment were explained to the study participants.

The inclusion criteria for the study were as follows: bone loss resulting from cyst removal, two-wall defects in most cases, three-wall defects, teeth affected by periodontal disease, extractions of impacted teeth, root caries, and crown fractures. The exclusion criteria were systemic diseases or endocrine/metabolic disorders that could affect the healing process (e.g. uncontrolled diabetes mellitus and hyperthyroidism), treatment with bisphosphonates or corticosteroids, cigarette smoking, chemotherapy, radiation therapy, pregnancy, acute or chronic odontogenic inflammation near the surgical site, and mental disorders.

Before the procedure, all individuals underwent professional dental hygienization, thorough dental checkups, and were orally administered antibiotics: clindamycin 0.6 g or amoxicillin/clavulanic acid 1 g. After surgery, pain medication (nimesil 100 mg) and a rinsing solution of chlorhexidine 0,1% were administered.

A total of 105 teeth were extracted and used as a biomaterial for bone reconstruction in the augmentation procedures (Figure 2). The extracted teeth were processed using three different devices: BonMaker (Korea Dental Solution Co. Ltd, Busan, Korea) in 13 patients, Tooth Transformer (TT Srl., Milan, Italy) in 13 patients, and Smart Dentin Grinder (KometaBio, Fort Lee, NJ, USA) in 13 patients.

Dentin matrix (both DDM and MDM) from ground natural teeth was used exclusively in the maxilla for all patients who underwent augmentation procedures. Histological examination was our main method for objective evaluation of the results. Cone-beam Computed Tomography (CBCT) scans were performed on all patients before and after treatment, as well as at the time of implant placement, using the Carestream CS 8200 3D system (Figure 3). Our study presents the following limitations: it was not possible to control some clinical variables, such as bone graft site conditions or type of dentin matrix used; the above mentioned drawbacks might have introduced a bias for the objective evaluation of the clinical outcomes. In our study, CBCT images were considered as a secondary testing parameter which couldn't be properly and statistically evaluated due to too many variables present for objective evaluation of the augmented area.

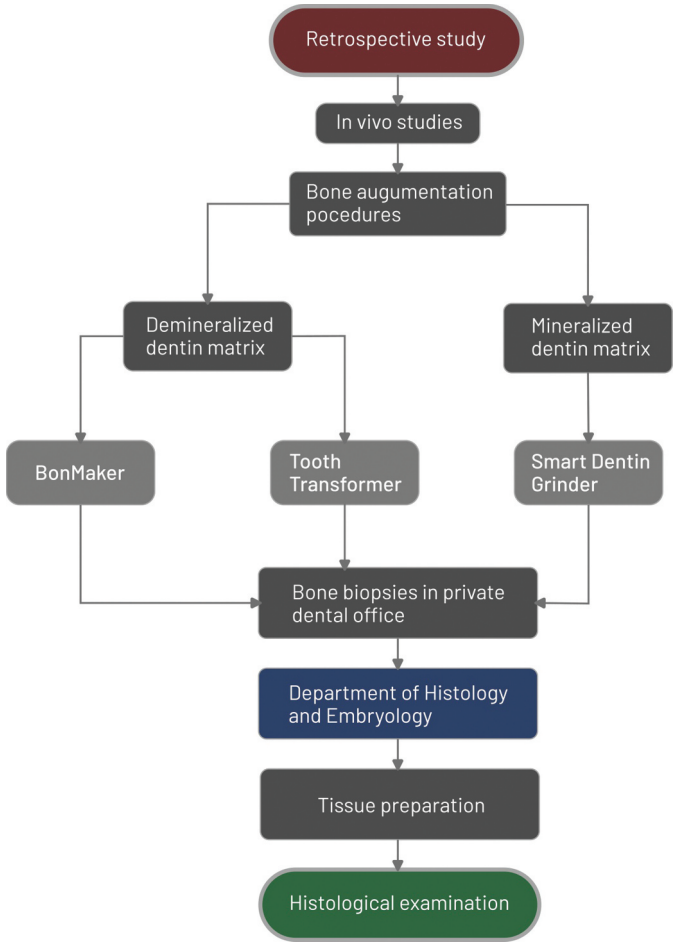

Figure 1. A flowchart of the study design.

Microbiological testing was performed on samples obtained from all three devices, and the results were negative for all samples. Histological examination of samples was conducted on patients from all groups, and the follow-up time ranged from 4 to 30 months.

Figure 2. Tooth grinders.

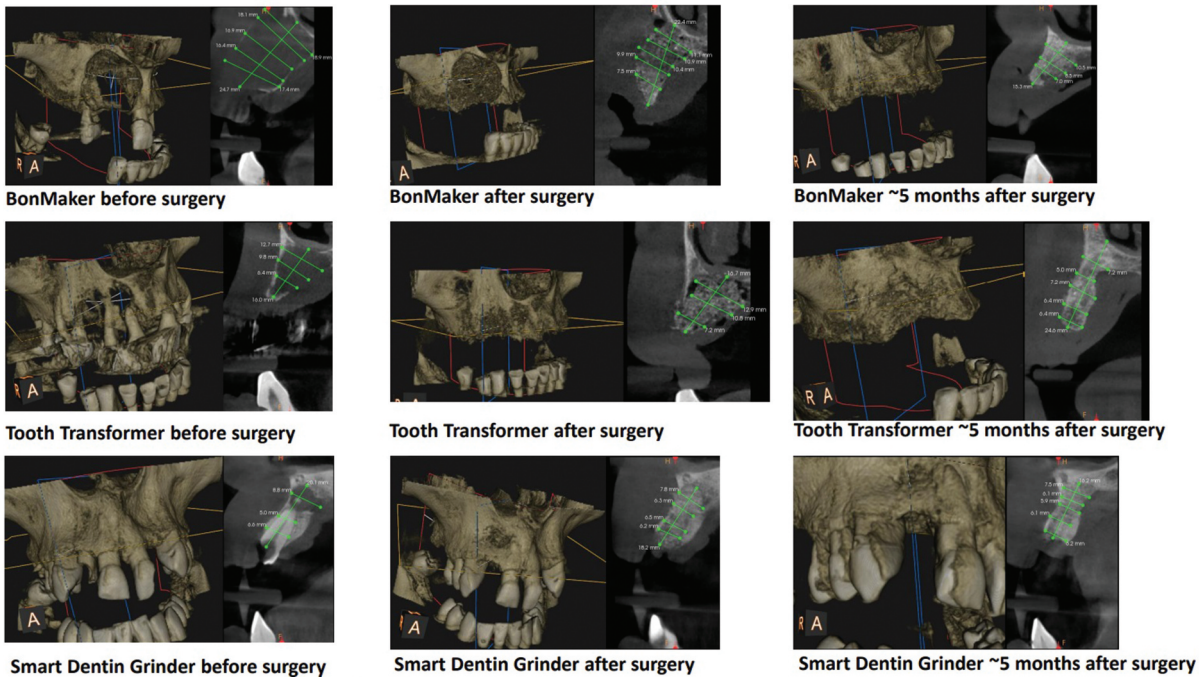

Figure 3. Examples of pre-operative and post-operative CBCT images of patients processed by different tooth grinders.

2.1.2. Graft preparation

The preparation of autogenous dentin grafts was carried out under the manufacturer's instructions for each device [13–15]. The general procedure for tooth preparation at DDC involved multiple steps. Firstly, the extracted tooth was immersed in a 3% hydrogen peroxide solution for 2–3 min to initiate decontamination. The tooth was then cleaned precisely using a diamond drill (e.g. Edenta 848 L) on a specialized isolated station (Figure 4) for tooth cleaning including: removal of soft tissues and tartar. After cleaning, the tooth was stripped of any fillings and discoloration of dentin and root cement. Enamel was removed from the teeth only in case of extensive caries or large fillings. Once prepared, the tooth was placed again in a 3% hydrogen peroxide solution for a few minutes, thoroughly dried, and transferred to a sterile mortar (Figure 5), where the teeth were crushed into smaller pieces. The crushed teeth were then transferred to one of the tooth grinders to obtain granules of 300–1200 µm, depending on the device, which is the optimal size for regenerating bone defects [16]. Finally, the teeth were processed into sterile pellets using one of the three devices where chemical sterilization takes place. In case of DDM the main demineralizing

Figure 4. Special desk for preparing teeth in DDC.

Figure 5. Trimmed and dried teeth inside surgical mortar before being placed in one of the mills.

Table 1. Clinical data, type of reagent, type of dentin matrix and DM processing time.

Method of DM preparation	Augmentation site in the maxilla (FDI numbering)	Main reagent	Type of dentin matrix	DM processing time
BonMaker (13 patients)	16,15,16,16–17,23–25,25–26,15–27,25–27,11–14,24,16,24,26	hydrochloric acid (HCL) with a concentration between 3,5–5%	demineralized dentin matrix	~20 minutes
Tooth Transformer (13 patients)	12,12,16,11–14,22–23,23–25,16–17,23–25,17–23,24–25,26,23	hydrochloric acid (HCL) with a concentration between 25–50%	demineralized dentin matrix	~30 minutes
Smart Dentin Grinder (13 patients)	16,25–26,13,17,21,14–15,16,24–26,23–26,12,14,25,24–27	Sodium hydroxide, 0.5 M solution	mineralized dentin matrix	~15–20 minutes

reagent used for both devices (BM and TT) was hydrochloric acid (HCL) of concentrations varying from 3–50%, while for MDM the main cleansing reagent was Sodium hydroxide, 0.5 M solution (Table 1).The selection of the device for each patient in this study was random and decided upon by the surgeon based on a physical and radiological examination of the patient. For the Smart Dentin Grinder, the mineralized protocol was followed.

2.1.3. Bone biopsies

All biopsies in the present study were obtained approximately 4–5 months after bone grafting with the DM in the maxilla, at the time of implant placement at Dłucik Dental Clinic by experienced surgeons. Samples for histological examination were obtained using a 4.0 Meisinger trephine bur (Figure 6) and were subsequently placed in a container filled with a formaldehyde solution for transport to the Department of Histology and Embryology, Medical University in Katowice, Poland.

2.2. In vitro studies

2.2.1. Tissue preparation

Upon receiving the samples, the extracted material was fixed in 10% formaldehyde and decalcified by immersing it for one week in TBD-1 Rapid Decalcifier, which contains EDTA and dilute hydrochloric acid. The decalcified samples were then subjected to the typical histological procedure of tissue dehydration using a graded ethanol series (ranging from 70 to 100%) at the Chandon Citadel 2000 automatic system. The dehydrated samples were embedded in paraffin blocks using the TEC-2800 Embedding Center, cut into 5 µm slices using the Microm HM 350S, and mounted onto slides. The slides underwent deparaffinization and rehydration and were then routinely stained with hematoxylin and eosin (HE) or Masson's trichrome [17]. Photographic documentation was performed using an Olympus BX43ZE microscope and Olympus Cell Sense software. The average sample size received from DDC was ~2 mm. The middle section of all samples was considered for histological examination.

3. Results

3.1. Histological examination

3.1.1. BonMaker (BM) (*Figures 7–9*)

Slides stained with standard (H-E) as well as Masson trichrome method show most of the dentin (D) encased in bone, where on the surface osteoblasts (black arrows) are observed. Inside the bone, osteocytes (white arrows) are singly enclosed within lacunae. Some ground dentin is surrounded by soft connective tissue (C). In addition, in Masson trichrome stained slides we can clearly delineate the boundaries between newly formed bone (blue NB) and already mineralized bone (red MB). No inflammation or other pathological structures were observed.

3.1.2. Tooth Transformer (TT) (*Figures 10–12*)

Both the H-E and Masson trichrome stained slides demonstrate that the fragments of ground dentin (D) have been effectively integrated into the jaw structure, with some being

Figure 6. Example of a sample extraction for histological examination by Meisinger trephine bur in the area of the maxillary first premolar.

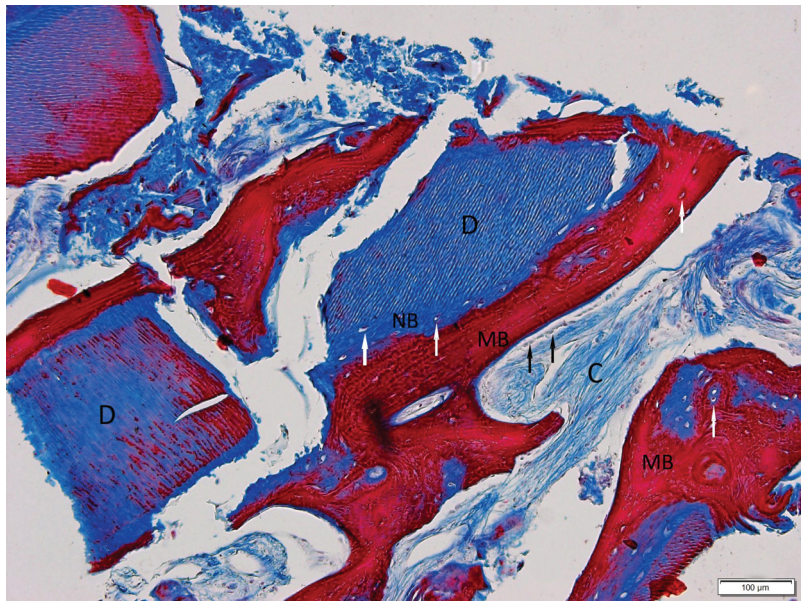

Figure 7. Sample stained with Masson trichrome – tissue from a patient processed by the BM. (D) dentin graft, (C) soft connective tissue, (NB) newly formed bone, and (MB) fully mineralized bone (white arrows: osteocytes, black arrows: osteoblasts). Objective magnification × 10.

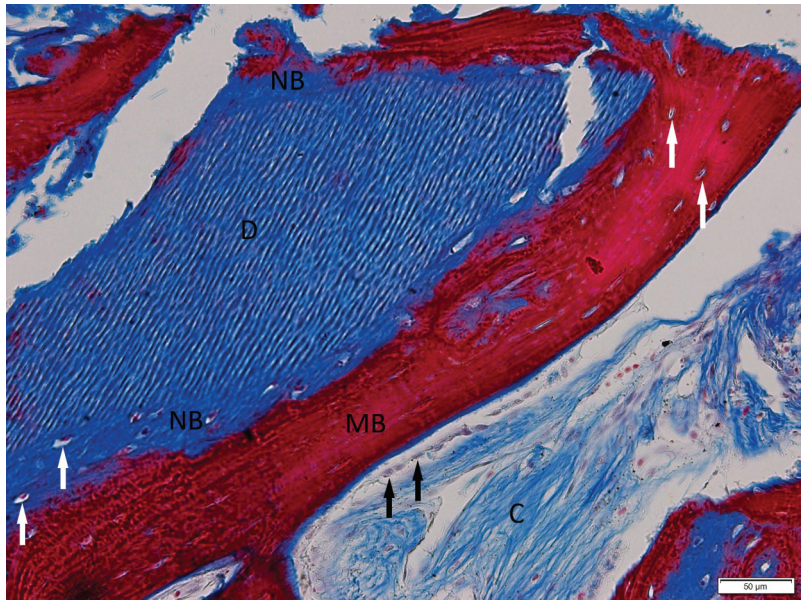

Figure 8. Tissue from a patient processed by the BM. An excellent connection between the dentin graft (D) and newly formed bone (NB) is visible in this sample. (C) soft connective tissue, (MB) fully mineralized bone, white arrows: osteocytes, black arrows: osteoblasts. Objective magnification × 20.

surrounded by soft connective tissue (C) while the majority are enclosed by newly forming bone (NB). This is particularly visible in the Masson trichrome stained slides, where the dark blue color of the new bone lamellae is directly adjacent to the dentin fragments (D). In the structure of newly formed bone, osteocytes and osteoblasts are clearly visible. None of the tissues show the presence of inflammation or any other pathological lesions.

3.1.3. Smart Dentin Grinder (SDG) (*Figures 13–15*)

Most dentin fragments (D) are surrounded by newly formed bone. Individual fragments remain surrounded by soft connective tissue (C). In both standard (H-E) and Masson trichrome stained slides, there are clearly visible osteocytes (white arrows) inside the bone tissue, and osteoblasts (black arrows) on its surface. Additionally, the images of the slides stained with Masson trichrome method display highlight the difference between newly formed bone (NB, blue) and fully mineralized bone (MB, red). No inflammation or other pathological structures were observed.

3.2. Evaluation of the healing process

In all 39 patients, the alveolar processes were successfully regenerated. In most cases, there were no significant complications during the recovery period that could affect the

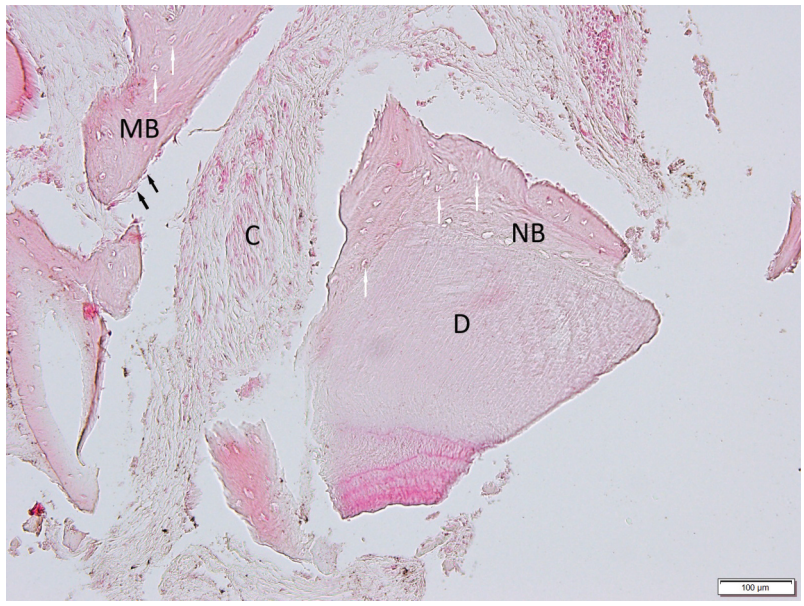

Figure 9. Sample stained with HE (BM). (D) dentin graft, (C) soft connective tissue, (NB) newly formed bone, and (MB) fully mineralized bone (black arrows: osteoblasts, white arrows: osteocytes). Objective magnification × 10.

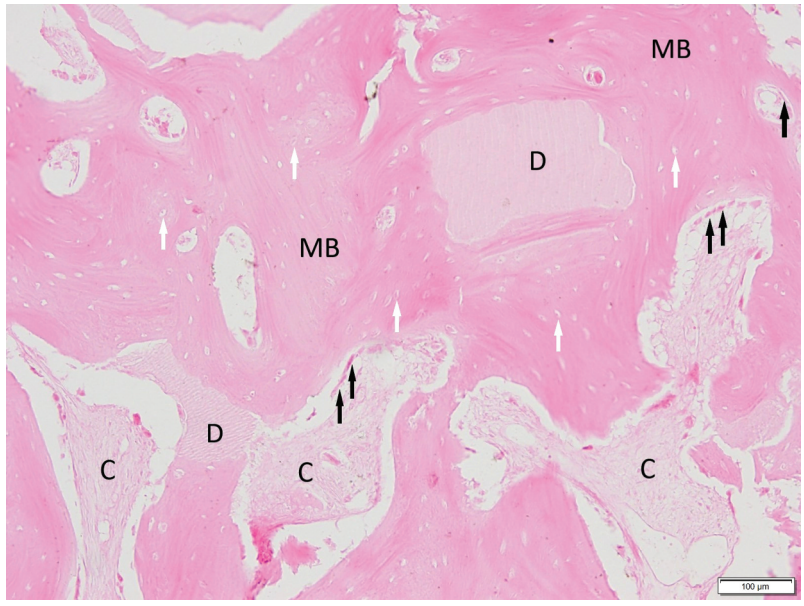

Figure 10. Tissue from a patient processed by the (TT), stained with HE. The dentin particle (D) is surrounded by bone (MB), and (C) soft connective tissue. Black arrows: osteoblasts, white arrows: osteocytes. Objective magnification × 10.

assessment of patient healing. In two cases, a hematoma developed as a consequence of the extraction of a lower impacted third molar to be used as augmentation material, but this did not affect the healing process. Additionally, none of the patients reported any other pain or discomfort.

Based on radiological (CBCT) examination, implantation could be performed after approximately 4 months in patients who underwent bone augmentation using the DM. During the follow-up period, we did not observe any marginal bone loss around implants that were inserted into bone augmented by DDM and MDM. For patients who underwent only augmentation procedures, no significant bone resorption was observed during the follow-up period, regardless of the method used.

4. Discussion

The devices for preparing autogenous dentin have been available on the global market for a considerable time; however, there is a dearth of studies in the literature that compare the healing outcomes of patients using all three methods in a single study. The histological examinations of our patients over the past few years have enabled us to make an objective comparison of each method.

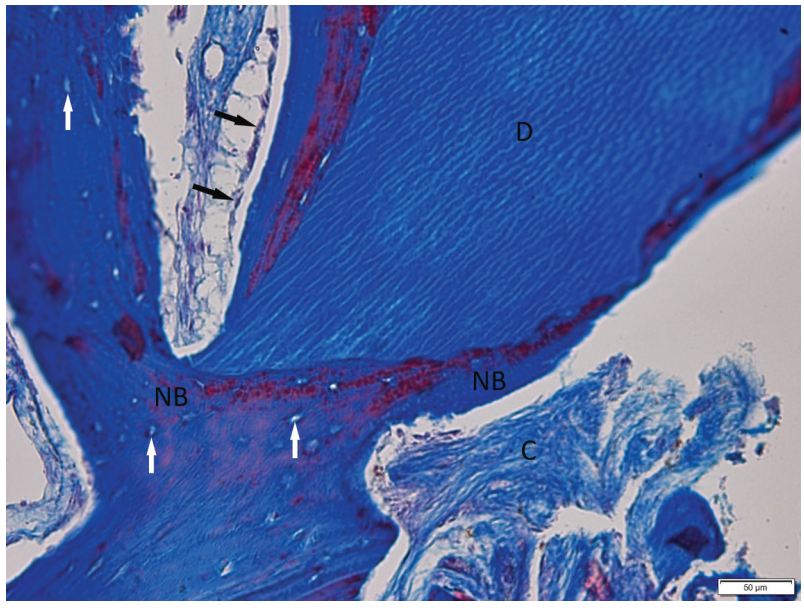

Figure 11. Sample processed by the TT, Masson trichrome staining. A perfect connection between the dentin graft (D) and new bone (NB) is visible here (black arrows: osteoblasts, white arrows: osteocytes), and (C) soft connective tissue. Objective magnification × 20.

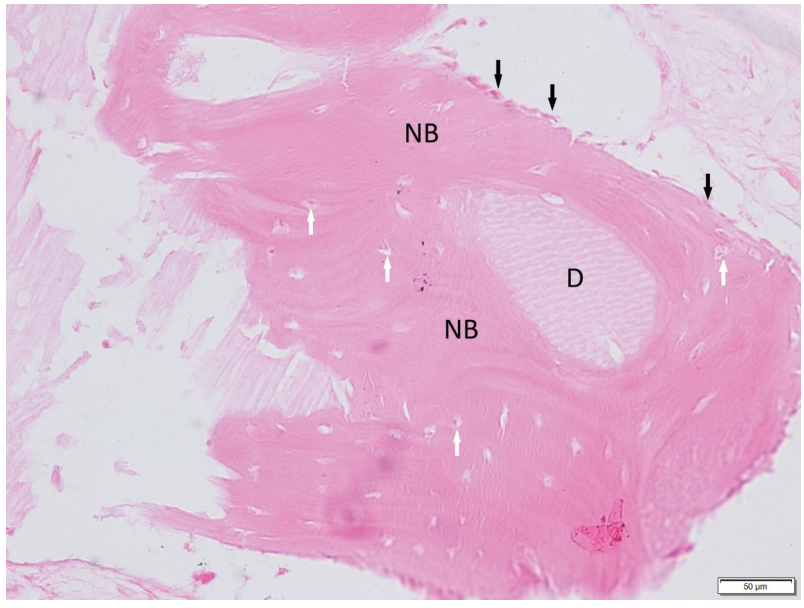

Figure 12. HE staining of a sample processed by the TT. The dentin graft (D) is well surrounded by new bone (NB). Osteocytes (white arrows) and osteoblasts (black arrows) are well visible here. Objective magnification × 20.

An ideal bone substitute should meet several conditions, including being osteoinductive, osteoconductive, osteogenic, biocompatible, and accessible [18]. Additionally, it should retain its shape over time as much as possible. Ground natural teeth-derived dentin matrix has recently emerged as a highly effective bone substitute material that meets these criteria and is obtained at a very low cost. In contrast, widely used xenografts, allogeneic grafts, and autogenous grafts are costly for both the patient and clinician and experience significant volume bioresorption over time [2,18,19]. The use of a dentin matrix does not require collagen membranes and perfectly preserves the shape of the regenerated outgrowth [13]. Furthermore, DM is biocompatible and undergoes slow volume resorption [20]. The effectiveness of using ground teeth as a bone substitute material to treat bone defects has been confirmed by recent histological studies in both animals and humans. In 2018 Dr Calvo-Guirado et al carried out histological and histomorphometric analysis of autogenous tooth grafts in dogs and their results indicated that tooth grafts can be considered an excellent bone graft material [14]. In 2021 Dr Radoczy-Drajko demonstrated with his histological results that Bonmaker autogenous tooth graft therapy may be safely and successfully used as a grafting

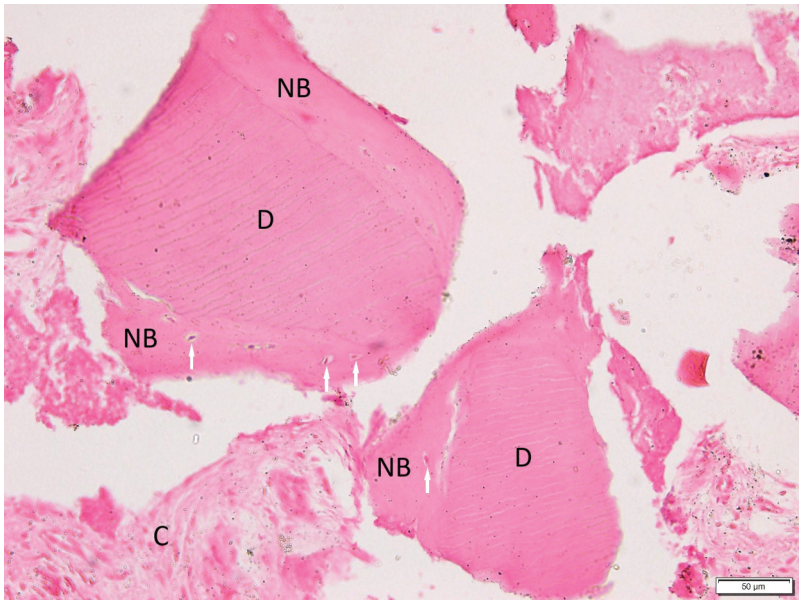

Figure 13. Sample processed by the SDG, HE staining. The dentin graft (D), new bone (NB), and osteocytes (white arrows) are well presented here. Objective magnification × 20.

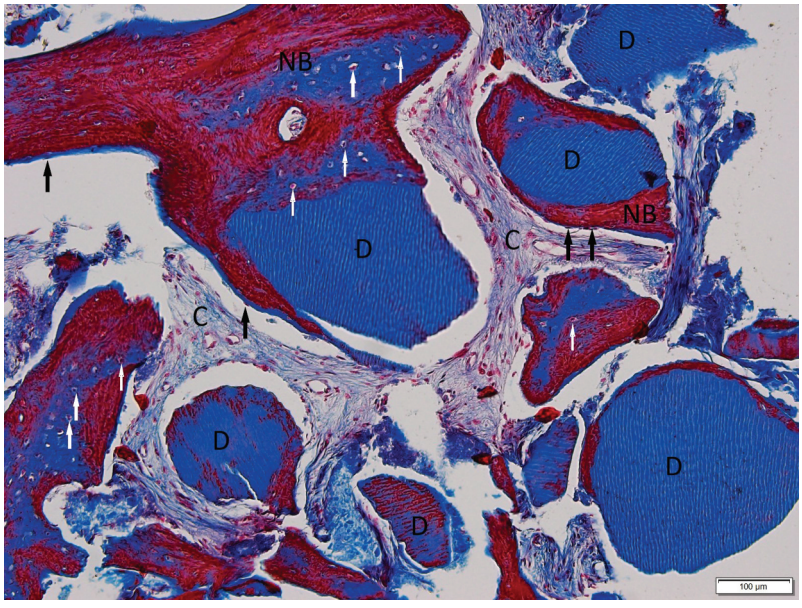

Figure 14. Many dentin grafts are visible (SDG). Some are already surrounded by bone tissue (NB, MD), while others are surrounded by soft connective tissue (C). Osteocytes (white arrows) and osteoblasts (black arrows) are present.Masson trichrome staining, objective magnification × 10.

material for alveolar ridge preservation [15]. In the same year Dr Cervera-Maillo et al suggested that particulate dentin grafts should be considered as an alternative material for sockets' preservation [9]. In a recent histological study in 2022, Dr Minetti et al proved that natural teeth could be considered as a source of bone substitute material [8]. Different methods were used to prepare granules from autogenous dentin, and clinical success was achieved with no inflammatory reactions or complications related to the augmentation procedures themselves. The researchers showed that dentin matrix is a biocompatible material that serves as a carrier for BMP-2, which significantly slows down alveolar atrophy [14,21–23]. Processing DM into sterile pellets takes up to 30 min, depending on the device, which provides many clinical opportunities in implant procedures [16]. Our histological findings are consistent with those of other studies, indicating the effectiveness of using ground teeth to treat bone defects. In addition no significant differences can be observed between the preparations obtained from patients who were augmented with dentin fragments obtained with different devices.

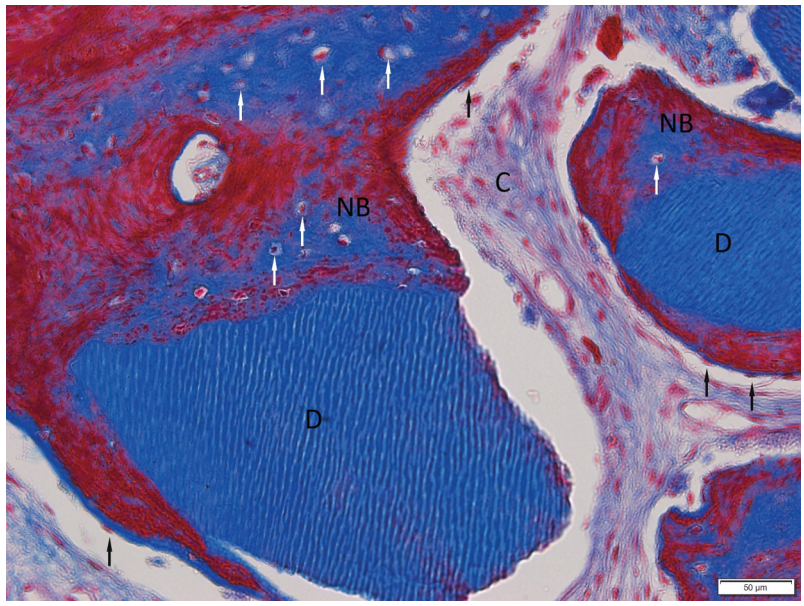

Figure 15. Sample processed by SDG. A very good connection between the dentin graft (D) and new bone (NB) is visible here (black arrows: osteoblasts on the surface, white arrows: osteocytes). (C) soft connective tissue. Masson trichrome staining, objective magnification × 20.

5. Conclusion

Histological examination of our patients allowed us to objectively compare all three devices in our practice. We concluded that all three systems are comparable, safe and highly effective in bone regenerative treatment. Both DDM and MDM give the same good results in alveolar bone reconstruction. We encourage other dentists and oral surgeons to consider using these devices for DM preparation in their practices as we believe it should become the new gold standard in oral implantation. However, further studies are necessary to confirm these data.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewers Disclosure

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

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