ceramics

MDPI

Review

Tooth-Derived Matrix Granules for Enhanced Bone Healing: Chemical Composition, Morphological Aspects, and Clinical Outcomes

Júlio C. M. Souza [1,2,3,*], Mario Escobar [4], Inês Sofia Pimentel [3], João Caramês [5], Wim Teughels [6], Filipe Silva [1,2] and Bruno Henriques [1,2,7]

1 Center for MicroElectroMechanical Systems (CMEMS-UMINHO), University of Minho, 4710-057 Braga, Portugal
2 Associate Laboratory (LABBELS), University of Minho, 4800-058 Guimarães, Portugal
3 University Institute of Health Sciences (IUCS), Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), 4585-116 Gandra, Portugal
4 Postgraduate Program in Dentistry (PPGO), Federal University of Santa Catarina (UFSC), Florianópolis 88040-900, Brazil
5 Bone Physiology Research Group, LIBPhys, School of Dentistry, Universidade de Lisboa, 1600-277 Lisbon, Portugal
6 Department of Oral Health Sciences, KU Leuven & Dentistry (Periodontology), University Hospitals Leuven, 3000 Leuven, Belgium
7 Ceramic and Composite Materials Research Group (CERMAT), Department of Mechanical Engineering (EMC), Campus Trindade, Federal University of Santa Catarina (UFSC), Florianópolis 88040-900, Brazil
* Correspondence: jsouza@dem.uminho.pt

check for updates

Citation: Souza, J.C.M.; Escobar, M.; Pimentel, I.S.; Caramês, J.; Teughels, W.; Silva, F.; Henriques, B. Tooth-Derived Matrix Granules for Enhanced Bone Healing: Chemical Composition, Morphological Aspects, and Clinical Outcomes. *Ceramics* **2022**, *5*, 981–990. https://doi.org/10.3390/ceramics5040070

Academic Editors: Paulo J. Palma and Antonio Riveiro

Received: 28 August 2022
Accepted: 8 November 2022
Published: 11 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Bone grafting has increasingly been used in surgical procedures for enhanced bone augmentation. Tooth-derived graft material has received considerable attention due to its chemical composition and autogenous source that can improve bone tissue healing. The main aim of this study was to provide a short and comprehensive review on the chemical composition, morphological aspects, and clinical outcomes of bone grafting using tooth-derived matrix granules. Dentin tissue has a chemical composition similar to that on bone tissues regarding the presence of hydroxyapatite, type I collagen, and different growth factors. Dentin-matrix granules are often processed at well-controlled size ranging from approximately 300 up to 1300 µm, while maintaining porosity and organic content. In addition, a dense collagen fiber network is still present after the milling and chemical treatment of dentin granules. Thus, dentin-matrix granules can improve the bone healing process considering their chemical composition, porous structure, and adequate size. However, further in vivo and in vitro studies should be performed taking into consideration different demineralization procedures, remnant organic content, porosity, and granule size.

Keywords: bone regeneration; bone grafting; dentin; chemical composition; tooth

1. Introduction

Restoration of alveolar ridge dimensions or bone reconstruction after tooth loss has been a challenge in implant dentistry. In some clinical cases, tooth forced eruption can be used as a tissue healing procedure and implant site development technique [1,2]. For instance, alveolar ridge preservation is a common clinical procedure performed to control alveolar ridge resorption after tooth extraction and to enable later implant placement and prosthetic rehabilitation [3]. Various types of bone substitutes have commonly been used for decades to restore the alveolar bone. Bone substitutes are classified according to their origin such as autogenous, allograft, xenograft, and alloplastic substitutes [4–8]. Autogenous bone is the first choice regarding its morphological aspects, chemical composition, and biologic content including osteogenic cells and growth factors [9–11]. Unfortunately, autogenous

bone has limitations related to its availability, morbidity, and remodeling process [12,13]. An adequate grafting material must provide a highly bioactivity and balanced resorption in association with proper mechanical properties that enable the preservation and healing of bone defects. Furthermore, the material should be cost-effective and easily handled at chair-side clinical cases [14].

In dentistry, teeth are often extracted due to several issues such as advanced periodontitis, caries, orthodontic treatment, or wisdom teeth (third molar) extraction indication. Nevertheless, alveolar ridge atrophy can occur as a consequence of gradual alveolar bone loss [5,15,16]. Previous studies have shown different strategies for alveolar atrophy reconstruction or ridge preservation by including autologous tooth matrix [17–20]. Experimental studies have provided evidence that autologous tooth-derived matrix (ATDM) reveals osteogenic stimuli thanks to its chemical composition and biological content [21–23]. The chemical composition of dentin is quite similar to that of bone tissues considering the presence of hydroxyapatite (60–80%), type I collagen, growth factors, and other proteins [21,24–26]. Dentin contains the following growth factors: insulin-like growth factor (IGF)-II, bone morphogenetic protein (BMP)-2, and transforming growth factor (TGF)-b [27–31].

Therefore, ATDM is considered as a chair-side bone substitute regarding its chemical composition and autologous source. Thus, a harvested tooth can be mechanically and chemically treated providing a particulate material within 15–20 min [32–34]. Mechanical debridement is performed by removing the enamel to expose the dentin and by grinding to produce granules [33,35]. Then, a chemical treatment is carried out to disinfect and remove debris, leading to the exposure of the collagen fiber content and release of proteins. Previous studies have reported the use of demineralizing substances for chemical treatment such as sodium hydroxide or hydrochloric acid, although different parameters have been assessed [32–34]. Thus, the clinical feasibility of preparing ATDM granules plays a major role in the use of such material as a bone substitute. However, the amount of ATDM granules is limited, and therefore, a mixture with xenogeneic or synthetic bone substitutes has become a promising strategy for bone healing. In addition, the source of graft material can be absent in some cases that do not show indications for tooth extraction. In fact, the type, concentration, and exposure time of the chemical substances determine the morphological aspects and chemical composition of the tooth-derived matrix. Clinical studies have revealed a higher density of bone formation around ATDM granules after implantation into bone defects as compared with control groups that are free of ATDM granules [36–39]. Such findings validate the use of ATDM granules as an alternative autologous bone substitute [21–23]. Nevertheless, correlations among chemical composition, morphological aspects, and bone formation have not been clear in previous studies, and therefore, bone formation can vary depending on the ATDM granules.

Thus, the main aim of the present study is to perform a concise review on the autologous tooth-derived matrix regarding preparation, chemical composition, and clinical evidence. It is hypothesized that tooth-derived matrix granules enhance interaction with proteins and cells leading to faster bone formation. The current review emphasizes the effects of the chemical composition and morphological aspects of autologous tooth-derived matrix on the clinical procedures for bone healing.

2. Method

A bibliographical search of articles was carried out on PubMed using the following search items: “tooth” OR “dentin” AND “granules” AND “chemical composition” OR “microscopy” OR “morphological” AND “bone healing” OR “bone regeneration” OR “bone repair”. The selection criteria involved articles published in the English language, within the last 15 years, that included in vitro, meta-analysis, and prospective cohort studies. Two of the authors (J.C.M.S. and M.E.R.) independently evaluated the titles and abstracts of potentially relevant studies. For this study, only papers that focused on chemical and morphological aspects of tooth-derived matrix granules were considered. The following variables were retrieved from the selected studies: authors’ names; publication

year, purpose of the study, chemical composition, chemical treatment, grinding process, microscopy, and in vivo outcomes.

3. Results and Discussion

Previous studies have reported the chemical composition and morphological aspects of ATDM granules and their effects on bone healing. The dentin-derived granules also reveal micro-scale pores that originate from the dentin tubules. The chemical and morphological factors play a key role in the interaction of ATDM granules with proteins and osteogenic cells that enhance the bone healing process. Thus, the results from previous in vitro and in vivo studies validate the hypothesis of the present study. A detailed discussion of previous findings is described below.

3.1. Chemical Composition and Morphological Aspects of Dentin-Derived Matrix

The chemical composition of bone and dentin are quite similar including hydroxyapatite (Hap) and type I collagen showing a high degree of cross-linking with matrix-binding proteins and growth factors such as IGF-II, BMP-2, and TGF [40,41]. Dentin-derived matrix granules are prepared from teeth which have been extracted due to trauma, advanced periodontal bone loss, or other indications such as wisdom teeth or orthodontic treatment. Immediately after extraction, restorative crowns and fillings must be cut off and removed as well as the tooth crown (Figure 1A). In addition, carious lesions, discolored dentin, periodontal ligament (PDL) remnants, and calculus should be removed by using tungsten carbide burs at high speed, according to previous studies [42,43].

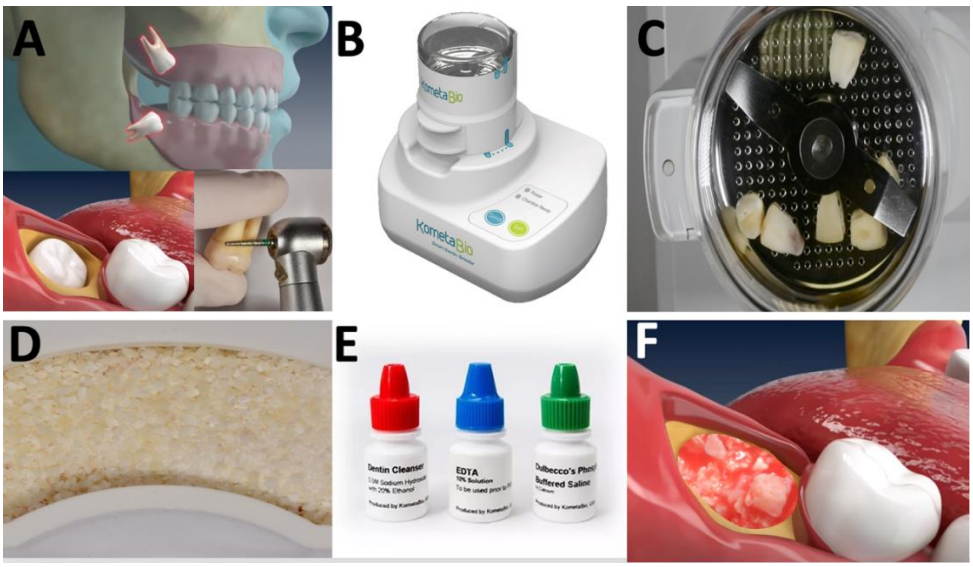

Figure 1. Schematics of the specimen preparation and clinical application: (**A**) Third molar extraction; (**B**) Smart Dentin Grinder^{TM} apparatus (KometaBio Inc., Cresskill, NJ, USA); (**C**) specimens in a sterile trituration chamber; (**D**) dentin granules with size ranging from 300 to 1200 µm; (**E**) cleaning and demineralizing solutions kit; (**F**) alveolar ridge preservation procedure.

For ATDM preparation, enamel and cementum are removed by using tungsten carbide burs exposing the dentin that is ground and chemically treated with disinfection solutions [44]. Tooth roots should be split out in the case of multi-rooted teeth. Then, the remnant tooth structures are dried using oil-free steam and ground using a grinder apparatus, as illustrated in Figure 1B,C. The grinding procedure can be designed to produce granules with size ranging from 300 up to 1200 µm in the collection tray, as seen in Figure 1D [32–34]. Thus, the grinding process is carried out for 3–5 s, and then granules are stirred for 20 s over sieves with different mesh size. Granules are separated by using different sized sieves. Particles with size below 300 µm fall into a waist drawer since they

do not show morphological features for bone grating. The grinding process can be repeated depending on the amount of tooth remnant.

The granules can be contaminated revealing a negligible risk of exposing patients to diseases, and therefore, an antibacterial chemical rinsing should be performed in solutions such as sodium hydroxide (NaOH), ethanol, and ethylenediaminetetraacetic acid (EDTA) (Figure 1E). The immersion time can vary depending on the protocol and chemical solutions. For instance, a rinsing procedure has been reported in NaOH and ethanol for 5 min [32–34]. Sodium hydroxide solution is a strong disinfection agent that has been used in several medical applications due to its defatting properties [33,34]. Sodium hydroxide is also effective in degrading proteins and nucleic acids as well as inactivating most viruses, bacteria, yeasts, fungi, and endotoxins. Ethanol is used in combination with NaOH to promote penetration of the disinfection solution into dentin tubules exposing the clean surface of the mineralized dentin matrix. Ethanol is safer than other disinfectants since it is rapidly eliminated, and the human body can also metabolize ethanol. As a second step, the application of EDTA, known as a chelating agent, has shown capability to remove bacteria by binding Mg^{2+} and Ca^{2+} ions from the outer cell wall of bacteria [34,45]. The antimicrobial effects of EDTA have been assessed for a wide range of microorganisms that include Gram-negative and Gram-positive bacteria, yeasts, ameba, and fungi. Moreover, EDTA is known to dissolve hydroxyapatite (Hap) from dentin l by chelating calcium, thus, exposing the dentin organic collagenous matrix and partially demineralizing the dentin granules [33,35]. After the EDTA treatment, granules are washed twice in sterile phosphate buffered saline (PBS). Then, PBS is decanted leaving wet particulate dentin ready for grafting into freshly extracted sockets or into alveolar bone defects, as shown in Figure 1F. The process from tooth extraction until grafting takes approximately 15–20 min. It should be noted that the efficiency of selecting the ATDM granules of specific size for grafting is higher than 95%. The volume of ATDM granules is more than twice of the original root volume. Alternatively, the wet ATDM granules can be dried over a hot plate (140 °C) for 5 min [2,33,34,46]. In fact, several protocols for the preparation of ATDM granules have been reported in the literature. Therefore, several other terms have been applied for ATDM depending on the processing protocols such as demineralized dentin-matrix (DDM), deproteinized demineralized dentin-matrix (dDDM), tooth-derived dentin-matrix (TDM), mineralized dentin-matrix (MDM), or partially mineralized dentin-matrix (PDM) [37,40,47].

Thus, the chemical treatment promotes a partial demineralization of the ATDM granules with the exposure of the collagen fibers and the release of dentin- derived growth factors and other Hap-binding proteins [28,48,49]. After immersion in demineralizing solutions, large dentin tubules and loosened collagen matrix may serve as channels for releasing proteins that are essential for proliferation and differentiation of osteogenic cells. ATDM granules reveal opened dentin tubules and porous surfaces that increase the adsorption and release of proteins in the surrounding surgical site [2]. An adequate balance in the demineralization process of ADTM granules should be achieved to provide a Hap-based matrix in combination with collagen fibers and growth factors. Furthermore, a partial demineralization procedure can be optimized for standard chemical composition and morphological aspects that enhance bone healing. Dentin tubules reveal diameters ranging from 1 up to 3 μm, and a proportion ranging from approximately 20 k to 50 k tubules/mm^2 can remain over dentin granules, as noticed in Figure 2. Depending on the chemical treatment, the size and spatial porous structure of ATDM granules can be enlarged, resulting in increased porosity from 3 up to 20% [50].

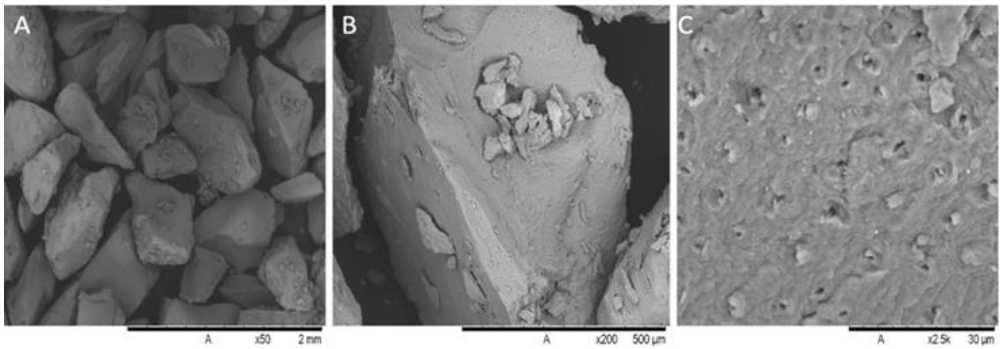

Figure 2. SEM images of ATDM granules at different magnifications: (**A**) 50×; (**B**) 500×; (**C**) 2500×.

The porous structure of ATDM granules increases their wettability and retains blood contents from host tissues including water, proteins, and minerals [51,52], that provides an optimal environment for the adhesion and proliferation of osteogenic cells. In fact, Hap still remains in the ATDM granules and the chemical composition and porous structure allow the release of growth factors [28,48,49]. Micro-scale porous surfaces stimulate the adhesion, proliferation, and differentiation of osteogenic cells leading to enhanced bone healing [51,53,54]. However, micro-scale pores at 1–3 μm do not allow cell infiltration and ingrowth or neither the formation of blood vessels. However, ATDM granules produced with size ranging from 300 to 1200 μm provide a large surface area for protein adsorption and cell adhesion for bone formation. The lack of macro-scale pores in the ATDM granules can slow down bone formation since the formation of blood vessels can not occur in the micro-scale pores of the granules. The ratio of granules in a bone defect determines the three-dimensional distribution of granules and enhancement of the biological events over the early period of bone healing [55]. Thus, the distance among the granules can be established by their amount and size distribution ranging from 300 up to 1200 μm. An increase in the amount of granules to fill a bone defect decreases the intergranule distance. An adequate inter-granule spacing is crucial for proper migration and differentiation of osteogenic cells, leading to the formation of blood vessels and new bone [55]. The formation of blood vessels takes place in the macro-scale spaces among particulate biomaterials with distance between 50 and 400 μm. A combination of granules and hydrogels has been studied to improve clinical handling of particulate bone graft materials and to control the distribution of granules in a bone defect [55–57]. Hydrogels (i.e., such as collagen- or fibrin-based materials) have been used to preserve a three-dimensional homogeneous distribution of graft material in a bone defect, maintaining adequate inter-granule spacing [55,56].

3.2. In Vivo Evidence of Bone Healing

Experimental in vivo studies have validated the biological performance of ADTM granules for bone healing. Several studies on the transplantation of human dentin blocks into human bone defects have shown noticeable intimate contacts without soft tissue that reveal progressive dentin-bone ankylosis after 6 months, and then replacement by bone tissue without any inflammatory reactions [58–60]. Thus, the feasibility of a clinical procedure with ATDM granules associated with their bone response has been receiving attention by scientists and clinicians [2,35,60]. Histological examinations of grafted sites from multiple studies have revealed the formation of a dentin–bone complex, where the tooth bone graft was surrounded by newly forming bone [61]. In addition, the histological results have shown remodeling processes between dentin and bone without signs of inflammatory reactions [2,21,30]. Cone beam computed tomography (CBCT) images have shown evidence of cortical and cancellous bone formation after 6 years follow-up [62–64]. A variable period of time required for resorption of dentin graft has been reported, and histologic studies have shown a characteristic depiction of graft particles encased in newly formed bone [8,60]. A lack of regular resorption time affects the predictably of the dentin-

based graft materials, and therefore, promotes obstacles in treatment planning, mainly in cases in which implants are required to be placed at the grafted sites.

Previous animal studies have reported bone formation in the tibia of rabbit and mice [23,45,65–67]. Many clinical studies have shown that new bone was formed by osteoinduction and/or osteoconduction on guided bone healing, socket preservation, sinus lift, and ridge augmentation [29,59,60,68–70]. Another case series study of 13 patients with 61 dentin-grafted post-extraction sockets compared CBCT images before and 4 months after surgery, and showed a gain in mid-palatal bone height (measured to the top of the graft still in the consolidation phase) and a loss of ridge width. A histological analysis revealed new bone formation in close contact to dentin particles with no signs of inflammatory reactions or fibrous encapsulation of the ATDM [14].

A prospective controlled clinical trial study evaluated the behavior of ATDM blocks during 26 weeks long-term follow-up observations based on 30 patients. Patients were divided into two groups who received ATDM blocks or autogenous bone blocks. The findings were consistent with those of previous short-term studies and indicated that ATDM blocks were capable of continuous remodeling under a functional load with appropriate volume maintenance, showing no statistical significance between both groups (p = 0.241) [26]. Regarding sinus lift procedures, a previous retrospective clinical study on the placement of 100 dental implants in 51 patients showed overall clinical success of 96.5%. A histologic examination showed that ATDM was gradually replaced by new bone to supporting the dental implants [71]. Hence, mineralized dentin became firmly integrated to the newly bone, establishing a solid site for anchorage of dental implants [60,70]. An alveolar ridge preservation technique with the use of ATDM graft has been associated with enhancing bone healing and maintaining bone volume for implant placement. Such previous findings support the strategy of using tooth-derived granules for repairing bone defects or preserving bone loss. Indeed, previous studies have revealed a gradual resorption rate of ATDM granules in late implant placement.

In the previous studies, several chair-side procedures have been described with variable use of chemical substances for partial demineralization of the ATDM granules. Variations in the content and exposure time for chemical treatment lead to different degrees of ATDM demineralization. In addition, the size of tubules and granules also varies depending on the physicochemical procedure, and therefore, further studies should be performed to optimize standard processing of ATDM granules. In addition, the availability of collagen fibers and growth factors in the dentin is also important for the migration and differentiation of osteogenic cells. A correlation between the processing and content of proteins should also be assessed in further studies. Regarding the clinical handling of particulate bone substitutes, the three-dimension distribution of granules in a bone defect cannot be controlled by a clinician. The use of hydrogels should be taken into consideration to provide an adequate inter-granule distance that results in enhanced bone healing. In this way, the combination of ATDM granules with hydrogels can become a promising clinical approach, and therefore, in vitro and in vivo studies should be performed to validate their clinical application.

4. Concluding Remarks

Within the limitations of the previous in vitro and in vivo studies, the main outcomes from the studies on autogenous tooth-derived dentin matrix can be drawn. Different guidelines for manufacturing autogenous tooth-derived dentin matrix have been reported in the literature depending on the grinding process and chemical treatment in demineralizing solutions. However, mean size of granules have been reported ranging from approximately 300 up to 1200 μm, which provided adequate surface area for bone formation and resorption rate with the replacement of granules by new bone. In addition, the dentin-derived granules possess an inherent micro-scale porous structure due to the presence of dentin tubules at 1–3 μm in diameter. Regarding chemical treatment with demineralizing solutions, the dentin tubules are enlarged and the mineral content composed of hydroxyapatite

is partially dissolved. Even though the granules are treated with demineralizing solutions, hydroxyapatite is still present and its porous structure allows the release of growth factors which stimulate the osteogenic cells in the bone healing process. Tooth-derived graft material has been shown to perform properly as expected for osteoconductive graft materials since an adequate amount of new bone has been histologically examined after a long healing time. Thus, autogenous tooth-derived dentin matrix disclosed structural, chemical, and biological aspects to serve as an alternative autologous bone substitute. In this context, it must be emphasized that autogenous tooth-derived dentin matrix appears to be clinically suitable for different reconstruction or preservation procedures for enhanced bone healing.

Author Contributions: Conceptualization, J.C.M.S. and B.H.; methodology, J.C.M.S. and B.H.; investigation, M.E., I.S.P. and J.C.M.S.; writing—original draft preparation, J.C.M.S., M.E., I.S.P. and B.H.; writing—review and editing, J.C.M.S., B.H. and J.C.; supervision, F.S., W.T., J.C. and J.C.M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Portuguese Foundation for Science and Technology (FCT) (POCI-01-0145-FEDER-031035_LaserMULTICER), SFRH/BPD/123769/ 2016, and CNPq-Brazil (CNPq/UNIVERSAL/421229/2018-7).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

References

1. Paolone, M.G.; Kaitsas, R. Orthodontic-periodontal interactions: Orthodontic extrusion in interdisciplinary regenerative treatments. *Int. Orthod.* **2018**, *16*, 217–245. [CrossRef] [PubMed]
2. Gideon Hallel, I.B. A Novel Procedure to Process Extracted Teeth for Immediate Grafting of Autogenous Dentin. *JBR J. Interdiscip. Med. Dent. Sci.* **2014**, *2*, 2–6. [CrossRef]
3. Park, S.-M.; Um, I.-W.; Kim, Y.-K.; Kim, K.-W. Clinical application of auto-tooth bone graft material. *J. Korean Assoc. Oral Maxillofac. Surg.* **2012**, *38*, 2. [CrossRef]
4. Becker, W.; Urist, M.; Becker, B.E.; Jackson, W.; Parry, D.A.; Bartold, M.; Vincenzzi, G.; De Georges, D.; Niederwanger, M. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. *J. Periodontol.* **1996**, *67*, 1025–1033. [CrossRef]
5. Hämmerle, C.H.F.; Araújo, M.G.; Simion, M. Evidence-based knowledge on the biology and treatment of extraction sockets. *Clin. Oral Implant. Res.* **2012**, *23*, 80–82. [CrossRef]
6. Artzi, Z.; Tal, H.; Dayan, D. Porous bovine bone mineral in healing of human extraction sockets. Part 1: Histomorphometric evaluations at 9 months. *J. Periodontol.* **2000**, *71*, 1015–1023. [CrossRef]
7. Valen, M.; Ganz, S.D. A synthetic bioactive resorbable graft for predictable implant reconstruction: Part one. *J. Oral. Implantol.* **2002**, *28*, 167–177. [CrossRef]
8. Kim, Y.-K.; Lee, J.; Yun, J.-Y.; Yun, P.-Y.; Um, I.-W. Comparison of autogenous tooth bone graft and synthetic bone graft materials used for bone resorption around implants after crestal approach sinus lifting: A retrospective study. *J. Periodontal Implant. Sci.* **2014**, *44*, 216–221. [CrossRef]
9. Urban, I.A.; Jovanovic, S.A.; Lozada, J.L. Vertical ridge augmentation using guided bone regeneration (GBR) in three clinical scenarios prior to implant placement: A retrospective study of 35 patients 12 to 72 months after loading. *Int. J. Oral Maxillofac. Implant.* **2009**, *24*, 502–510.
10. Dłucik, R.; Orzechowska-Wylęgala, B.; Dłucik, D.; Puzzolo, D.; Micali, A. Socket preservation or guided bone regeneration—A case report. *Pol. Merkur. Lek.* **2021**, *49*, 153–157.
11. Minetti, E.; Giacometti, E.; Gambardella, U.; Contessi, M.; Ballini, A.; Marenzi, G.; Celko, M.; Mastrangelo, F. Alveolar Socket Preservation with Different Autologous Graft Materials: Preliminary Results of a Multicenter Pilot Study in Human. *Materials* **2020**, *13*, 1153. [CrossRef] [PubMed]
12. Mendoza-Azpur, G.; de la Fuente, A.; Chavez, E.; Valdivia, E.; Khouly, I. Horizontal ridge augmentation with guided bone regeneration using particulate xenogenic bone substitutes with or without autogenous block grafts: A randomized controlled trial. *Clin. Implant. Dent. Relat. Res.* **2019**, *21*, 521–530. [CrossRef]

13. von Arx, T.; Cochran, D.L.; Hermann, J.S.; Schenk, R.K.; Buser, D. Lateral ridge augmentation using different bone fillers and barrier membrane application. A histologic and histomorphometric pilot study in the canine mandible. *Clin. Oral Implant. Res.* **2001**, *12*, 260–269. [CrossRef]
14. Pohl, S.; Binderman, I.; Tomac, J. Maintenance of Alveolar Ridge Dimensions Utilizing an Extracted Tooth Dentin Particulate Autograft and PlateletRich Fibrin: A Retrospective Radiographic ConeBeam Computed Tomography Study. *Materials* **2020**, *13*, 1083. [CrossRef] [PubMed]
15. Araujo, M.G.; Lindhe, J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J. Clin. Periodontol.* **2005**, *32*, 212–218. [CrossRef] [PubMed]
16. Mezzomo, L.A.; Shinkai, R.S.; Mardas, N.; Donos, N. Alveolar ridge preservation after dental extraction and before implant placement: A literature review. *Rev. Odonto Ciênc.* **2011**, *26*, 77–83. [CrossRef]
17. Esposito, M.; Maghaireh, H.; Grusovin, M.G.; Ziounas, I.; Worthington, H.V. Interventions for replacing missing teeth: Management of soft tissues for dental implants. *Cochrane Database Syst. Rev.* **2012**, CD006697. [CrossRef]
18. Giesenhagen, B.; Martin, N.; Donkiewicz, P.; Perić Kačarević, Ž.; Smeets, R.; Jung, O.; Schnettler, R.; Barbeck, M. Vertical bone augmentation in a single-tooth gap with an allogenic bone ring: Clinical considerations. *J. Esthet. Restor. Dent.* **2018**, *30*, 480–483. [CrossRef]
19. Maeda, D.; Lima, F.; Meza, J.; Ciotti, D.L.; Mizutani, F.S.; Doyle, H.; Faveri, M. Alveolar Ridge Regeneration of Damaged Extraction Sockets using a Bovine-derived Bone Graft in Association with a Titanium Foil: Prospective Case Series. *J. Int. Acad. Periodontol.* **2020**, *22*, 109–116.
20. Qiu, L.; Yu, H. Onlay grafting with bovine bone mineral block for horizontal reconstruction of severely atrophic alveolar ridges in anterior maxillae: A 6-year prospective study. *J. Craniomaxillofac. Surg.* **2018**, *46*, 1199–1204. [CrossRef]
21. Cardaropoli, D.; Nevins, M.; Schupbach, P. New Bone Formation Using an Extracted Tooth as a Biomaterial: A Case Report with Histologic Evidence. *Int. J. Periodontics Restor. Dent.* **2019**, *39*, 157–163. [CrossRef] [PubMed]
22. Andersson, L.; Ramzi, A.; Joseph, B. Studies on dentin grafts to bone defects in rabbit tibia and mandible; development of an experimental model. *Dent. Traumatol. Off. Publ. Int. Assoc. Dent. Traumatol.* **2009**, *25*, 78–83. [CrossRef] [PubMed]
23. Bormann, K.H.; Suarez-Cunqueiro, M.M.; Sinikovic, B.; Kampmann, A.; von See, C.; Tavassol, F.; Binger, T.; Winkler, M.; Gellrich, N.C.; Rücker, M. Dentin as a suitable bone substitute comparable to ß-TCP–an experimental study in mice. *Microvasc. Res.* **2012**, *84*, 116–122. [CrossRef] [PubMed]
24. Brudevold, F.; Steadman, L.T.; Smith, F.A. Inorganic and Organic Components of Tooth Structure. *Ann. N. Y. Acad. Sci.* **1960**, *85*, 110–132. [CrossRef] [PubMed]
25. Leonhardt, Å.; Dahlén, G.; Renvert, S. Five-Year Clinical, Microbiological, and Radiological Outcome Following Treatment of Peri-Implantitis in Man. *J. Periodontol.* **2005**, *74*, 1415–1422. [CrossRef] [PubMed]
26. Schwarz, F.; Becker, J.; Hazar, D.; Becker, K.; Sader, R.; Becker, J. Efficacy of autogenous tooth roots for lateral alveolar ridge augmentation and staged implant placement. A prospective controlled clinical study. *J. Clin. Periodontol.* **2018**, *45*, 996–1004. [CrossRef]
27. Dragoo, M.R.; Kaldahl, W.B. Clinical and histological evaluation of alloplasts and allografts in regenerative periodontal surgery in humans. *Int. J. Periodontics Restor. Dent.* **1983**, *3*, 8–29.
28. Cenicante, J.; Botelho, J.; Machado, V.; Mendes, J.J.; Mascarenhas, P.; Alcoforado, G.; Santos, A. The use of autogenous teeth for alveolar ridge preservation: A literature review. *Appl. Sci.* **2021**, *11*, 1853. [CrossRef]
29. Hussain, I.; Moharamzadeh, K.; Brook, I.M.; José de Oliveira Neto, P.; Salata, L.A. Evaluation of osteoconductive and osteogenic potential of a dentin-based bone substitute using a calvarial defect model. *Int. J. Dent.* **2012**, *2012*, 396316. [CrossRef]
30. Yüceer-Çetiner, E.; Özkan, N.; Önger, M.E. Effect of Autogenous Dentin Graft on New Bone Formation. *J. Craniofac. Surg.* **2021**, *32*, 1354–1360. [CrossRef] [PubMed]
31. Graziano, A.; d'Aquino, R.; Laino, G.; Papaccio, G. Dental pulp stem cells: A promising tool for bone regeneration. *Stem Cell Rev.* **2008**, *4*, 21–26. [CrossRef] [PubMed]
32. Calvo-Guirado, J.L.; Maté-Sánchez de Val, J.E.; Ramos-Oltra, M.L.; Pérez-Albacete Martínez, C.; Ramírez-Fernández, M.P.; Maiquez-Gosálvez, M.; Gehrke, S.A.; Fernández-Domínguez, M.; Romanos, G.E.; Delgado-Ruiz, R.A. The Use of Tooth Particles as a Biomaterial in Post-Extraction Sockets. Experimental Study in Dogs. *Dent. J.* **2018**, *6*, 12. [CrossRef]
33. Calvo-Guirado, J.L.; Ballester Montilla, A.; De Aza, P.N.; Fernández-Domínguez, M.; Gehrke, S.A.; Cegarra-Del Pino, P.; Mahesh, L.; Pelegrine, A.A.; Aragoneses, J.M.; Maté-Sánchez de Val, J.E. Particulated, Extracted Human Teeth Characterization by SEM^{-}EDX Evaluation as a Biomaterial for Socket Preservation: An in vitro Study. *Materials* **2019**, *12*, 380. [CrossRef] [PubMed]
34. Calvo-Guirado, J.; Garcés-Villalá, M.; Mahesh, L.; De Carlos-Villafranca, F. Effectiveness of chemical disinfection in discarding pathogenic bacteria of human particulate tooth graft: An In vitro study. *Indian J. Dent. Sci.* **2021**, *13*, 277. [CrossRef]
35. Santos, A.; Botelho, J.; Machado, V.; Borrecho, G.; Proença, L.; Mendes, J.J.; Mascarenhas, P.; Alcoforado, G. Autogenous Mineralized Dentin versus Xenograft granules in Ridge Preservation for Delayed Implantation in Post-extraction Sites: A Randomized controlled clinical trial with an 18 months follow-up. *Clin. Oral Implant. Res.* **2021**, *32*, 905–915. [CrossRef]
36. Mazzucchi, G.; Lollobrigida, M.; Lamazza, L.; Serafini, G.; Di Nardo, D.; Testarelli, L.; De Biase, A. Autologous Dentin Graft after Impacted Mandibular Third Molar Extraction to Prevent Periodontal Pocket Formation-A Split-Mouth Pilot Study. *Materials* **2022**, *15*, 1431. [CrossRef]

37. Catanzaro-Guimarães, S.A.; Catanzaro Guimarães, B.P.; Garcia, R.B.; Alle, N. Osteogenic potential of autogenic demineralized dentin implanted in bony defects in dogs. *Int. J. Oral Maxillofac. Surg.* **1986**, *15*, 160–169. [CrossRef]
38. Radoczy-Drajko, Z.; Windisch, P.; Svidro, E.; Tajti, P.; Molnar, B.; Gerber, G. Clinical, radiographical and histological evaluation of alveolar ridge preservation with an autogenous tooth derived particulate graft in EDS class 3-4 defects. *BMC Oral Health* **2021**, *21*, 63. [CrossRef]
39. Li, P.; Zhu, H.; Huang, D. Autogenous DDM versus Bio-Oss granules in GBR for immediate implantation in periodontal postextraction sites: A prospective clinical study. *Clin. Implant Dent. Relat. Res.* **2018**, *20*, 923–928. [CrossRef]
40. Tanwatana, S.; Kiewjurat, A.; Suttapreyasri, S. Chemical and thermal deproteinization of human demineralized tooth matrix: Physicochemical characterization and osteoblast cell biocompatibility. *J. Biomater. Appl.* **2019**, *34*, 651–663. [CrossRef]
41. Bono, N.; Tarsini, P.; Candiani, G. Demineralized dentin and enamel matrices as suitable substrates for bone regeneration. *J. Appl. Biomater. Funct. Mater.* **2017**, *15*, e236–e243. [CrossRef] [PubMed]
42. Lamont, T.; Worthington, H.V.; Clarkson, J.E.; Beirne, P.V. Routine scale and polish for periodontal health in adults. *Cochrane Database Syst. Rev.* **2018**, *12*, CD004625. [CrossRef] [PubMed]
43. Muduroglu, R.; Ionescu, A.C.; Del Fabbro, M.; Scolavino, S.; Brambilla, E. Distribution of adhesive layer in class II composite resin restorations before/after interproximal matrix application. *J. Dent.* **2020**, *103*, 103494. [CrossRef] [PubMed]
44. Murata, M.; Okubo, N.; Shakya, M.; Arafat Kabir, M.; Yokozeki, K.; Zhu, B.; Ishikawa, M.; Kitamura, R.; Akazawa, T. Dentin Materials as Biological Scaffolds for Tissue Engineering. In *Biomaterial-Supported Tissue Reconstruction or Regeneration*; IntechOpen: London, UK, 2019; p. 13.
45. Farzad, P.; Lundgren, T.; Al-Asfour, A.; Andersson, L.; Dahlin, C. Integration of Dental Implants in Conjunction with EDTA-Conditioned Dentin Grafts: An Experimental Study. *Dent. J.* **2021**, *9*, 63. [CrossRef] [PubMed]
46. Minamizato, T.; Koga, T.; Takashi, I.; Nakatani, Y.; Umebayashi, M.; Sumita, Y.; Ikeda, T.; Asahina, I. Clinical application of autogenous partially demineralized dentin matrix prepared immediately after extraction for alveolar bone regeneration in implant dentistry: A pilot study. *Int. J. Oral Maxillofac. Surg.* **2018**, *47*, 125–132. [CrossRef]
47. Koga, T.; Minamizato, T.; Kawai, Y.; Miura, K.I.; Takashi, I.; Nakatani, Y.; Sumita, Y.; Asahina, I. Bone regeneration using dentin matrix depends on the degree of demineralization and particle size. *PLoS ONE* **2016**, *11*, e0147235. [CrossRef]
48. Um, I.-W.; Lee, J.-K.; Kim, J.-Y.; Kim, Y.-M.; Bakhshalian, N.; Jeong, Y.K.; Ku, J.-K. Allogeneic Dentin Graft: A Review on Its Osteoinductivity and Antigenicity. *Materials* **2021**, *14*, 1713. [CrossRef]
49. Pai, G.P.; Dayakar, M.M.; Sreedhar, A.S. Dentin Graft—The Hidden Treasure. *Int. J. Res. Eng. Sci. Manag.* **2021**, *4*, 19–21.
50. Pashley, D.H. Dentin: A dynamic substrate—A review. *Scanning Microsc.* **1989**, *3*, 161–176.
51. Feng, S.; Li, R.; Wang, Z. Experimental study on the biocompatibility and osteogenesis induction ability of PLLA/DDM scaffolds. *Odontology* **2022**, *110*, 508–522. [CrossRef]
52. Goldberg, M.; Kulkarni, A.B.; Young, M.; Boskey, A. Dentin: Structure, composition and mineralization. *Front. Biosci. Elite* **2011**, *3*, 711–735. [CrossRef] [PubMed]
53. Brennan, C.M.; Eichholz, K.F.; Hoey, D.A. The effect of pore size within fibrous scaffolds fabricated using melt electrowriting on human bone marrow stem cell osteogenesis. *Biomed. Mater.* **2019**, *14*, 065016. [CrossRef] [PubMed]
54. Reddi, A.H.; Huggins, C.B. Influence of Geometry of Transplanted Tooth and Bone on Transformation of Fibroblasts. *Exp. Biol. Med.* **1973**, *143*, 634–637. [CrossRef] [PubMed]
55. Palma, P.J.; Matos, S.; Ramos, J.; Guerra, F.; Figueiredo, M.H.; Kauser, J. New formulations for space provision and bone regeneration. *Biodental Eng. I* **2010**, *1*, 71–76.
56. Noronha Oliveira, M.; Varela, H.A.; Caramês, J.; Silva, F.; Henriques, B.; Teughels, W.; Quirynen, M.; Souza, J.C.M. Synergistic Benefits on Combining Injectable Platelet-Rich Fibrin and Bone Graft Porous Particulate Materials. *Biomed. Mater. Devices* **2022**. [CrossRef]
57. Cortellini, S.; Castro, A.B.; Temmerman, A.; Van Dessel, J.; Pinto, N.; Jacobs, R.; Quirynen, M. Leucocyte- and platelet-rich fibrin block for bone augmentation procedure: A proof-of-concept study. *J. Clin. Periodontol.* **2018**, *45*, 624–634. [CrossRef]
58. Matsuzawa, Y.; Okubo, N.; Tanaka, S.; Kashiwazaki, H.; Kitagawa, Y.; Ohiro, Y.; Mikoya, T.; Akazawa, T.; Murata, M. Primary Teeth-Derived Demineralized Dentin Matrix Autograft for Unilateral Maxillary Alveolar Cleft during Mixed Dentition. *J. Funct. Biomater.* **2022**, *13*, 153. [CrossRef]
59. Ramanauskaite, A.; Sahin, D.; Sader, R.; Becker, J.; Schwarz, F. Efficacy of autogenous teeth for the reconstruction of alveolar ridge deficiencies: A systematic review. *Clin. Oral Investig.* **2019**, *23*, 4263–4287. [CrossRef]
60. Andrade, C.; Camino, J.; Nally, M.; Quirynen, M.; Martínez, B.; Pinto, N. Combining autologous particulate dentin, L-PRF, and fibrinogen to create a matrix for predictable ridge preservation: A pilot clinical study. *Clin. Oral Investig.* **2020**, *24*, 1151–1160. [CrossRef]
61. Um, I.-W.; Ku, J.-K.; Kim, Y.-K.; Lee, B.-K.; Leem, D.H. Histological Review of Demineralized Dentin Matrix as a Carrier of rhBMP-2. *Tissue Eng. Part B Rev.* **2020**, *26*, 284–293. [CrossRef]
62. Kim, Y.K.; Lee, J.; Um, I.W.; Kim, K.W.; Murata, M.; Akazawa, T.; Mitsugi, M. Tooth-derived bone graft material. *J. Korean Assoc. Oral Maxillofac. Surg.* **2013**, *39*, 103–111. [CrossRef]
63. Kim, S.Y.; Kim, Y.K.; Park, Y.H.; Park, J.C.; Ku, J.K.; Um, I.W.; Kim, J.Y. Evaluation of the Healing Potential of Demineralized Dentin Matrix Fixed with Recombinant Human Bone Morphogenetic Protein-2 in Bone Grafts. *Materials* **2017**, *10*, 1049. [CrossRef] [PubMed]

64. Kim, Y.-K.; Pang, K.-M.; Yun, P.-Y.; Leem, D.-H.; Um, I.-W. Long-term follow-up of autogenous tooth bone graft blocks with dental implants. *Clin. Case Rep.* **2017**, *5*, 108–118. [CrossRef] [PubMed]
65. Jin, S.-C.; Kim, S.-G.; Oh, J.-S.; Lee, S.-Y.; Jang, E.-S.; Piao, Z.-G.; Lim, S.-C.; Jeong, M.-A.; Kim, J.-S.; You, J.-S.; et al. A comparative study of bone formation following grafting with different ratios of particle dentin and tricalcium phosphate combinations. *J. Biomed. Nanotechnol.* **2013**, *9*, 475–478. [CrossRef] [PubMed]
66. Gharpure, A.S.; Bhatavadekar, N.B. Clinical Efficacy of Tooth-Bone Graft: A Systematic Review and Risk of Bias Analysis of Randomized Control Trials and Observational Studies. *Implant Dent.* **2018**, *27*, 119–134. [CrossRef]
67. Al-Asfour, A.; Andersson, L.; Kamal, M.; Joseph, B. New bone formation around xenogenic dentin grafts to rabbit tibia marrow. *Dent. Traumatol. Off. Publ. Int. Assoc. Dent. Traumatol.* **2013**, *29*, 455–460. [CrossRef]
68. Kim, Y.-K.; Lee, J.-H.; Um, I.-W.; Cho, W.-J. Guided Bone Regeneration Using Demineralized Dentin Matrix: Long-Term Follow-Up. *J. Oral Maxillofac. Surg.* **2016**, *74*, 515.e1–515.e9. [CrossRef]
69. Cervera-Maillo, J.M.; Morales-Schwarz, D.; Morales-Melendez, H.; Mahesh, L.; Calvo-Guirado, J.L. Autologous Tooth Dentin Graft: A Retrospective Study in Humans. *Medicina* **2021**, *58*, 56. [CrossRef]
70. Valdec, S.; Pasic, P.; Soltermann, A.; Thoma, D.; Stadlinger, B.; Rücker, M. Alveolar ridge preservation with autologous particulated dentin-a case series. *Int. J. Implant Dent.* **2017**, *3*, 12. [CrossRef]
71. Jeong, K.I.; Kim, S.G.; Kim, Y.K.; Oh, J.S.; Jeong, M.A.; Park, J.J. Clinical study of graft materials using autogenous teeth in maxillary sinus augmentation. *Implant Dent.* **2011**, *20*, 471–475. [CrossRef]