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Dentin-derived bone graft for bone healing

Integrative sistematic review

Paula R. Navarro Fernández

Tese conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

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Trabalho realizado sob orientção do Prof Doutor Julho Souza

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a minha mãe por falar comigo as vezes que quis renunciar, quando senti que não podia continuar.

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Abstract

Purpose: The main aim of this study was to perform an integrative review on the effect of the dentin matrix graft for enhanced bone healing.

Method: A bibliographic review was performed on PubMed using the following search terms: "dentin" OR "tooth-derived" AND "particle" OR "granule" AND "bone healing" OR "bone repair" OR "bone regeneration" OR "osteoblast". Studies published in English language until February 28th, 2022 were selected regarding the purpose of this study.

Results: The bibliographic search resulted in 23 selected studies in human participants, animals, cell culture, and laboratory research. The chemical treatment of dentin matrix granules involved immersion in different reactive substances such as NaOH or HCl, or HNO_3 for partially demineralization of the dentin matrix exposing the collagen fibers, opening the dentin tubules' diameter, and releasing growth factors. The presence of hydroxyapatite, type I collagen fibers, and proteins (i.e., BMP-2) was detected on the rough surfaces and porous structure of dentin matrix granules. A high proliferation and differentiation of osteogenic cells over dentin matrix granules was recorded in cell culture assays. A higher amount of new bone around dentin matrix granules was recorded in bone defects when compared to non-grafted surgical sites. The amount of new bone was comparable to the sites grafted with demineralized bovine bone mineral.

Conclusions: The chemical composition and rough/porous morphological aspects of dentin matrix granules can provide a high bioactivity inducing the migration and adhesion of proteins and osteogenic cells when placed in bone defects. In vivo studies revealed a formation of new bone around dentin matrix granules validating their potential use as alternative bone substitute.

Key terms: tooth-derived matrix, demineralized dentin matrix, bone healing, osteoblast

Resumo

Objective: O objetivo principal deste estudo foi realizar uma revisão integrativa sobre o efeito dos grânulos de dentina usados como enxertos para reparação óssea.

Método: Uma revisão bibliográfica foi realizada na PubMed usando os seguintes termos de busca: *"dentin" OR "tooth-derived" AND "particle" OR "granule" AND "bone healing" OR " bone repair" OR "bone regeneration" OR "osteoblast".*

Resultados: A busca bibliográfica resultou em 23 estudos selecionados tendo em vista trabalhos *in vivo* e *in vitro*. O tratamento químico dos grânulos da matriz dentinária envolveu a imersão em diferentes substâncias reativas como NaOH ou HCl, ou HNO_3 para desmineralização parcial da matriz dentinária expondo as fibras de colagénio, túbulos dentinários e fatores de crescimento. Hidroxiapatite, fibras de colagénio tipo I e proteínas (ex. BMP-2) foram detectados nas superfícies e na estrutura porosa dos grânulos da matriz dentinária. Uma alta proliferação e diferenciação de células osteogênicas foi registrada em ensaios de cultura de células. Um maior volume de tecido ósseo formado ao redor dos grânulos da matriz dentinária em defeitos ósseos foi detectado quando comparados aos sítios cirúrgicos não enxertados. A quantidade de tecido formado foi comparável aos locais enxertados com mineral ósseo bovino desmineralizado.

Conclusões: A composição química e os aspectos morfológicos dos grânulos da matriz dentinária proporcionam uma alta bioatividade induzindo a migração e adesão de proteínas

e células osteogênicas após implantação em defeitos ósseos. Estudos in vivo revelaram a formação de tecido ósseo ao redor dos grânulos da matriz dentinária validando seu potencial uso como substituto ósseo alternativo.

Key terms: tooth-derived matrix, demineralized dentin matrix, bone healing, osteoblast.

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1. Introduction

Several bone substitutes have been used to repairing alveolar defects to gathering bone volume and stability for further placement of dental implants or prosthetic structures (1–5). Bone substitutes are characterized considering the source for manufacturing that involves autologous, allogenous, xenogenous, or synthetic (alloplastic) materials (1,3,6,7). Among the bone substitutes, autografts such as autologous bone tissues are the first choice for bone healing regarding the chemical composition including the presence of proteins and growth factors (7,8). Nevertheless, autogenous tooth-derived grafts have getting attention considering the re-use of extracted teeth such as third molars or premolars withing orthodontic treatment planning (9–11).

Extracted teeth are source of hydroxyapatite, collagen type I, and proteins (i.e., BMP-2) at similar proportions when compared to bone tissues (12,13). However, the preparation of tooth-derived graft is not a standard procedure since several protocols are reported in literature. Recent procedures recommend the use of dentin and therefore the enamel and cementum of extracted teeth are removed. Then, dentin is milled by using an automatic grinder apparatus to manufacturing of granules with size ranging from approximately 250 up 1,200 µm. Granules are chemically treated by different substances (i.e, NaOH, HNO_3, HCl) and then rinsed in distilled water and phosphate buffered solutions prior to sterilization procedures (9,14,15). The chemical treatment of dentin granules is required for conditioning the crystalline hydroxyapatite although the chemical reaction depends on the chemical composition of the solutions and immersion time. Smear layers and most of mineral phase are eliminated from the surfaces of the granules and dentin tubules providing the exposure of the collagen fibers' network and release of proteins like growth factors (12). As a result, dentin granules treated with chemical agents possess rough surfaces and open dentin tubules that increase the surface area for interaction with proteins and osteogenic cells when placed in surgical sites. The adhesion of proteins, minerals, and osteogenic cells over

rough surfaces and porous materials is higher when compared to smooth surfaces and non-porous materials (16–18). Additionally, that stimulate the migration and differentiation of osteogenic cells leading to the formation a collagen matrix and adsorption of calcium and phosphorous and then enhancing the mineralization process and bone formation around the rough and porous surfaces.

Several other terms have been used for tooth-derived graft materials 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), partially mineralized dentin matrix (PDM) (9,12,14). Thus, the tooth-derived graft intends to provide particulate materials containing mineral compounds based on calcium and phosphate embedding proteins and type I collagen for enhanced bone healing. Nevertheless, physicochemical analyses and biocompatibility assays (*in vitro* and *in vivo*) should clarify the biological effects of current types of tooth-derived grafts for different clinical cases considering an enhanced bone healing.

2. Objetives and hyphotesis

The main aim of this study was to perform an integrative review on the effect of the dentin matrix graft granules for an enhanced bone healing. It was hypothesized that the chemical composition and morphological aspects of dentin matrix graft granules induce the migration of osteogenic cells leading to an enhanced bone formation.

3. Methods

3.1 Information sources and search strategy

A bibliographic review was performed on PubMed (via National Library of Medicine) considering such database includes the major articles in the field of dentistry and biomaterials. The present method was performed in accordance with the search strategy applied in previous studies on integrative or systematic reviews (16,19–22). The following search terms were applied: "dentin" OR "tooth-derived" AND "particle" OR "granule" AND "bone healing" OR "bone repair" OR "bone regeneration" OR "osteoblast". Also, a hand-search was performed on the reference lists of all primary sources and eligible studies of this systematic review for additional relevant publications. The inclusion criteria encompassed articles published in the English language, reporting the chemical composition and morphological aspects of the human dentin matrix graft mineral to inducing bone healing. The eligibility inclusion criteria used for article searches also involved: *in vitro* studies; meta-analyses; randomized controlled trials (RCT); animal assays; and prospective cohort studies. The exclusion criteria were the following: papers without abstract and case report with short follow-up period. Studies based on publication date were not restricted during the search process.

3.2 Study selection and data collection process

The selection of studies was carried out into three steps. At first, studies were scanned for relevance by title, and the abstracts of those that were not excluded at this stage were assessed. Three of the authors (JCMS, PRF) independently analyzed the titles and abstracts of the retrieved, potentially relevant articles meeting the inclusion criteria. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager. The second step comprised

the evaluation of the abstracts and non-excluded articles, according to the eligibility criteria on the abstract review. Selected articles were individually read and analyzed concerning the purpose of this study. At last, the eligible articles received a study nomenclature label, combining first author names and year of publication. The following variables were collected for this review: authors' names, journal, publication year, aims, morphological aspects, chemical composition, and main outcomes on the bone healing. PICO question was adjusted to the issue where "P" was related to the patients or specimens while "I" referred to the methods of analyses. Data of the reports were harvested directly into a specific data-collection form to avoid multiple data recording regarding multiple reports within the same study (e.g., reports with different set-ups). Such evaluation was individually carried out by two researchers, followed by a joint discussion to select the relevant studies.

4. Results

The initial search on PubMed database identified a total of 135 studies of which 17 duplicates were removed, as shown in Figure 1. The titles and abstracts of the 118 studies were read seeking concordance with the inclusion criteria of the present review study. A total of 92 studies were removed concerning they did not meet the inclusion criteria. The evaluation of titles and abstracts resulted in 26 potentially relevant studies, although 3 studies were excluded because they did not provide comprehensive data taking into account the purpose of this review study. At last, 23 studies were included in this review (Figure 1).

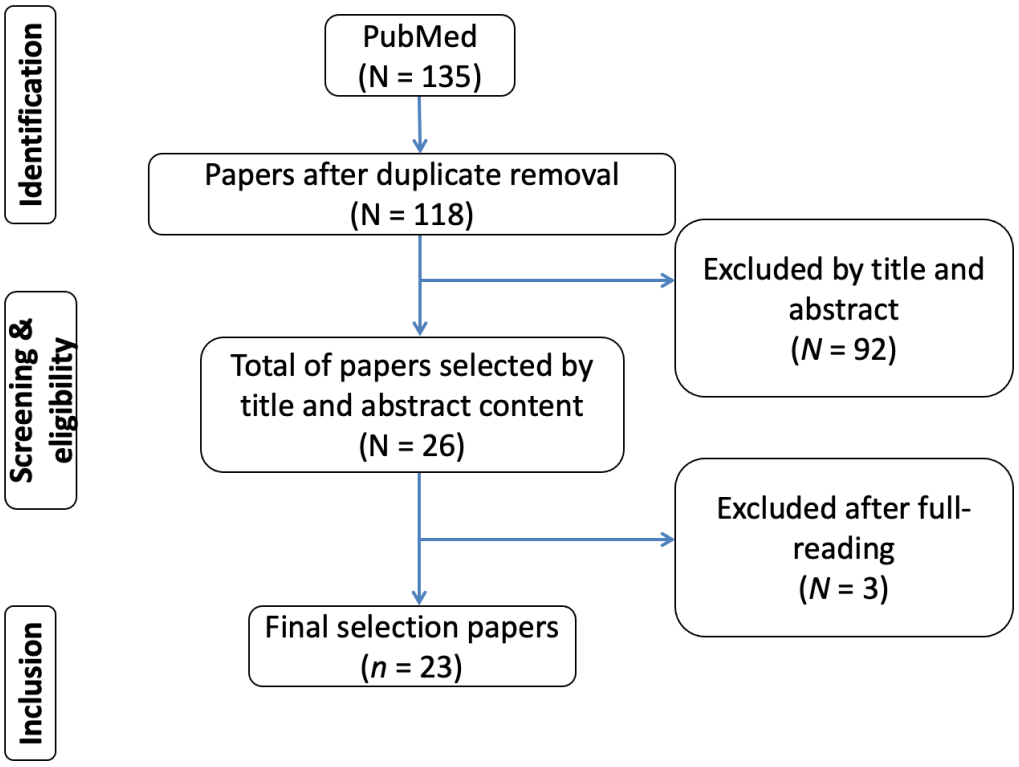

Figure 1. Flow chart on the identification and selection of studies.

Within the 23 selected studies, 19 studies reported the effects of dentin matrix graft (DMG) granules in human participants or animals while 4 studies were performed only *in vitro*. Regarding in vivo studies, 9 studies reported histomorphometry results after placement of DMG granules in bone defects of human participants (9–11,23–28) while 8 studies reported histomorphometry results after placement DMG granules in tissue defects of animals (14,29–34). One study showed CBCT images of the new bone formation around TDM granules after placemen in bone defects of human participants while 3 studies revealed micro-CT images after placement of DMG granules in bone defects of animals (14,28,31,32). Three studies reported the effects of the TDM granules on the primary stability of dental implants. Six studies also involved high-resolution microscopic analyses such as using scanning electron microscopy (SEM) coupled to energy dispersive spectroscopy (EDS) although 4 studies were performed only in laboratory (12,13,29,35,36). Regarding the preparation of DMG granules, two studies assessed different substances and immersion time, of which one study also assessed the effect of a thermal treatment for manufacturing deproteinized DMG granules. Regarding *in vitro* studies, 4 studies assessed the behavior of osteogenic cells in contact with DMG granules. Data on the study design, methods, and main outcomes gathered from major relevant studies are shown in Table 1. The major results reported in the selected studies are describes as follow.

- Dentin matrix granules were milled using different apparatus such as: Smart Dentin Grinder™, (KometaBio Inc., USA); Bonmaker™ (Korea dental solution, Korea); Mixer Mill M301™ (Retsch GmbH, Germany); Osteo-Mill (Tokyo Iken Co Ltd, Japan), Transformer TT™ (TT Transformer S.r.l, Italy), and Korean Tooth Bank device (Seoul, Korea) (Table 1). The tested granules revealed a minimum size at 200 μm (14) and maximum at 1,500 μm (28) although a range from 300 up to 1200 μm was assessed in most of studies, as seen in Table 1;
- Several procedures involving a chemical treatment prior to rinsing in PBS and distilled water were assessed on *in vivo* and *in vitro* studies such as: (i) immersion in 0.5 M NaOH and 20% (v/v) ethanol; (ii) immersion in in 0.5 M NaOH and 30% (v/v) alcohol for 5 min; (iii) Immersion in 0.5M HCl for 3 h at 25 °C; (iv) immersion

in in 2% HNO_3 for 20 min; (v) disinfection in 5% peracetic acid and 75% ethanol for 10 min. The type of chemical substance and time of immersion determined the degree of demineralization of the granules (12,14). Even though granules were treated by demineralization substances, hydroxyapatite with ration at 1.5-1.8 was detected as the mineral phase of the granules (12,13). Also, collage type I and BMP-2 were detected after chemical treatment procedures (13). Cytocompatibility assays revealed a high proliferation of osteogenic cells over DMG granules (12,13);

- A previous *in vitro* study performed a thermal treatment for deproteinization of the tooth-derived granules (12). SEM images showed open dentinal tubules with smooth dentin surfaces after thermal treatment while rough dentin surface was revealed after immersion in NaOH followed by thermal treatment (12);
- Clinical studies on X-Rays or CBCT imaging revealed a higher density of bone formation around DMG granules after implantation into bone defects when compared with control groups free of DMG granules (9,11,15,28). Histomorphometry findings also revealed a high amount of new bone around DMG granules (9,26,28,34). Core biopsies showed DMG granules surrounded by 56% newly formed bone and connective tissues (28). The percentage of newly formed bone around dentin-derived particles (~47%) was significantly higher than that recorded for DBBM xenograft (~35%) ($p<.001$), and the proportion of residual graft was significantly lower (12%) around dentin-derived particles for 18-months follow up (9);
- Dental implants placed after 6-motnhs bone grafting with dentin matrix granules or DBBM xenograft revealed similar primary stability (72-77) (9,11,27) and secondary implant stability (~80-81) (9). There was no statistically significant difference between both groups in implant stability quotient values and marginal bone resorption.

Table 1. Data gathered from the selected studies.

Author (Year), Country	Purpose	Study design	Milling processing/ Granules' size (μm)	Analyses	Main outcomes
Ku et al (2022), Korea	Evaluating the effects of 15 and 25 kGy Gamma radiation on the osteoinductive properties of demineralized dentin matrix at extra-skeletal sites	In vivo (animal study) Demineralized dentin matrix particles were implanted in subcutaneous tissues of the dorsal thigh muscles of 20 nude mice	Extracted teeth were processed at Korean Tooth Bank (Seoul, Korea). Milled dentin particles (300–800 μm) were ultrasonically cleaned in distilled water and then dehydrated with	Dual-energy X-ray absorptiometry Alkaline phosphatase (ALP) assays Histology on tartrate-resistant acid phosphatase (TRAP) staining	New bone formation was identified in all the groups at each time point. In conclusion, Gamma radiation at doses of 15 and 25 kGy does not affect the osteoinductive properties of demineralized dentin matrix.

			ethyl alcohol and defatted using ethyl ether solution. Granules were then demineralized for 30 min in 0.6 N HCl. The demineralized particles were lyophilized, packed, and sterilized with ethylene oxide gas.		
Mazzucchi et al (2022), Italy	Evaluating the efficacy of an autologous dentin graft in preventing	In vivo (case reports). 6 months follow-up Extraction sockets from 10 human participants	Milled dentin granules (300 to 1200 μm) were immersed into a	Radiografic analysis: Periapical X-ray	The measurements recorded at six months showed a reduction of the probing pocket depth

	periodontal defects after impacted or semi-impacted lower third molars surgical extraction.	(split-mouth) were filled with autologous dentin graft, while the control sites were filled with blood clot. Post-extractive sites were monitored at 15, 90 and 180 days.	0.5 M NaOH and 20% ethanol solution and then rinsed twice with PBS		distal to the second lower molar (M2) at both surgical sites. Radiographic evaluation also showed a greater amount of bone gain at the grafted sites compared to the control sites.
Santos et al (2021), Portugal	Evaluating the primary stability of delayed implants placed in post-extraction ridges preserved with autogenous mineralized dentin	RCT. 18 months follow-up 52 human participants requiring ridge preservation in preparation for delayed implant placement in post-extraction sites.	Milling extracted teeth root using a Smart Dentin Grinder™, (KometaBio Inc., USA) Particles (250-1,200 µm)	Cone beam computed tomography (CBCT). A resonance frequency analyser (Osstell IDx Mentor, Osstell AB, Sweden)	MDM granules and xenograft groups promoted similar primary (~ 77) and secondary (80-81) implant stability. The percentage of newly formed bone around MDM granules (~47%) was

	matrix (MDM) versus xenograft granules. Clinical, histological and pain experience outcomes were further assessed.	After 6 months, trephine cores were harvested for histomorphometry prior to implant placement. Implants were then placed, and implant stability was measured immediately as well as two months after placement. Marginal bone loss and presence of mucositis/peri-implantitis were recorded up to 18 months after prosthetic loading.	immersed in 0.5 M NaOH and 30% (v/v) alcohol for 5 min and then in PBS for two quick rinses. PBS was carefully removed with sterile gauze, and the material was kept in room temperature	was used to record implant stability Digital periapical radiographs were made via VistaScan image plate scanner (Durr Dental AG, Bietigheim-Bissingen, Germany) Haematoxylin and eosin staining Patient's pain and discomfort perceptions were rated for 7 days after the allocated intervention via visual	significantly higher than that for xenograft (~35%) ($p < .001$), and the proportion of residual graft was significantly lower (12% around MDM granules. No significant differences were found as far as clinical, radiographic and patient-related outcomes.

				analogue scale (VAS) score (0–10)	
Radoczy-Drajko et al (2021), Hungary	Clinical, radiographical, and histological evaluation of the safety and efficacy of autogenous tooth Bonmaker powder in the treatment postextraction sockets with alveolar ridge preservation	In vivo (case reports). 6 months follow-up A total of 9 teeth were extracted from 5 human patients. The extraction sockets were filled up with autogenous tooth particulate.	Milling enamel-free extracted teeth using a Bonmaker™ device (Korea dental solution, Korea). Particles (425-1,500 μm) were disinfected for 20min following the manufacturer's instruction.	CBCT Intraoral x-rays Core biopsies and hematoxylin and eosin staining for histomorphometry assays	Core biopsies showed autogenous tooth particles surrounded by 56% newly formed bone and connective tissue. Only a mean of 7% of non-remodeled autogenous tooth particles was recorded.
Minetti et al (2020),	Histological and histomorphometrical evaluation comparing	Case reports. 4 months follow-up	Extracted teeth were automatically	Histological preparation and fuchsin/blue toluidine	Autologous grafts surrounded by new bone were recorded in all

Italy	vital whole and non-vital endodontically treated teeth used as autologous grafts in post-extractive socket preservation procedures	23 human participants with post-extractive defects were divided into two groups considering the use of endodontically-treated teeth or not. membrane for socket preservation. After 4 months, 32 bone biopsies were harvested for histomorphometry analysis.	processed using a tooth transformer device to produce granulated graft material used with a collagen membrane porcine pericardium (Bego oss™)	staining for histomorphometry assays.	samples and partially resorbed dentin and enamel structures were detected.
Tanwatana et al (2019), Thailand	Developing a deproteinized human demineralized tooth matrix to be used as a	*In vitro* study. Deproteinization of demineralized tooth matrix was performed via three protocols; (a)	Caries-free third molar and premolar teeth. Tooth was carefully cleaned,	Chemical analyses: XRD, XFS, EDS, FTIR, Microscopy: SEM Cell culture assays: Resazurin based	SEM showed open dentinal tubules with smooth dentin surfaces for thermal and H_2O_2/thermal group, while

	bone graft substitution	thermal treatment, (b) NaOH/thermal treatment and (c) H_2O_2/thermal treatment Cell culture in contact with osteoblasts (MC3T3-E1) for 1,3,5,7, and 14 days.	divided into crown and root portion. Pulp and periodontal tissue were removed. Tooth was milled in granules using a mixer ball mill machine (Mixer Mill M301™, Retsch GmbH, Germany). Sieves with 500 µm and 1000 µm aperture (Endecotts, London, UK) were used to select desired particle size range from	(PrestoBlue™) cell viability. Spectrophometry at 570 nm	NaOH/thermal group showed rough dentin surface. XRD revealed only hydroxyapatite phase. XFS detected Ca and P and a Ca/P ratio at 1.5-1.8. Osteoblasts attached and grew on the NaOH/thermal deproteinized human demineralized tooth matrix.

			500 to 1000 µm. A partially demineralization of tooth granules was performed in 0.5M HCl for 3 h at 25 oC.		
Li et al (2018), China	Evaluating the clinical efficacy of autogenous dentin derived graft versus Bio-Oss™ granules for immediate implantation in periodontal postextraction sites.	Prospective clinical study. 18-months follow up. Forty human participants were randomly allocated into two groups: Placement of autogenous dentin derived graft or Bio-Oss™ (Geistlich Pharma	Dentine was grinded by an automatic mill (Osteo-Mill™, Tokyo Iken Co Ltd, Japan) at 20 000 rpm for 7-10 s. Granules (from 300 to 1200 µm) were partially demineralized in	Implant stability quotient (ISQ) was measured by Osstell Mentor (Integration Diagnostics AB, Sweden) Digital periapical radiograph of the graft site taken with paralleling technique or panoramic	There was no statistically significant difference between both groups in implant stability quotient values and marginal bone resorption. Autogenous dentin-derived granules prepared at the chairside after extractions could act as an excellent readily available

		AG, Switzerland) cancellous granules.	2% HNO_3 for 20 min to expose the dentine's organic matrix and then disinfected in 5% peracetic acid and 75% ethanol for 10 min to remove any bacteria and smear layer (defatting and sterilization). At last, dentin granules were washed twice with distilled water.	radiograph was performed immediately, at 6 and 18 months after surgery.	alternative to bone graft material

Pang K (2017), Korea	Evaluate the clinical efficacy and histological of the autogenous tooth graft (AutoBT) compared to anorganic bovine bone in post-extraction alveolar bone augmentation.	A prospective RCT. 6-months follow up. A total of 33 graft sites in 24 human participants. 21 bone defect sites of 15 patients were grafted using AutoBT™ while anorganic bovine bone was placed in 12 defects of 9 patients for alveolar bone augmentation 2–4 weeks after dental extraction .	Extracted teeth were processed at Korean Tooth Bank (Seoul, Korea). Milled dentin granules (300 and 800 μm) were washed, defatted, decalcified, lyophilized, and sterilized with ethylene oxide. Graft material was stored at room temperature for clinical use.	Primary stability of implant fixture was recorded using Osstell Mentor Resonance Frequency Analyser (Osstell AB, Sweden). Histological preparation for histomorphometry assays. Quantitative evaluations of ratio of newly formed bone volume compared to total volume.	The vertical dimensions of alveolar bone increased by ~5.3 mm in AutoBT group and ~6.5 mm in anorganic bovine bone group at 6 months post-extraction. Histomorphometrically, new bone formation of AutoBT-grafted site was ~31 % while that of Bio-Oss" was ~35%. The implant stability quotient (ISQ) of implants placed in AutoBT-grafted sites was measured at 72 for AutoBT-grafted and 70 for anorganic bovine bone-grafted sites. There were

					no statistically significant differences between measurements of the two groups.
Bono et al (2017), Italy	Investigating the effects of demineralization on the physical-chemical and biological behavior of dentin and enamel particles.	In vitro Human dentin and enamel granules were processed for physicochemical analyses and cell culture assays	Teeth were milled sing a Tooth Transformer TT (TT Transformer S.r.l, Italy) producing granules (< 1 mm). Granules were (i) treated with demineralization reagent (reagent A) at 70°C under shaking at 1,000 rpm; (ii) washed sequentially with 2	SEM-EDS ELISA assays for determining mineral, collagen type I and BMP-2 Cell culture in contact with MG-63 and SAOS-2 osteogenic cells for 3 and 7 days. Viability evaluation by Alamar blue assays.	The chemical treatment of dentin granules allowed preserving the collagen content, while increasing BMP-2 bioavailability. Enamel granules showed a high content of mineral phase.

			solutions (reagents B and C) for 2 min; (iii) treated with sterilization reagent (reagent D) at 70°C under shaking at 1,000 rpm. Granules were finally washed with reagent E and for 2 min.		
Koga et al (2016), Japan	Evaluating the influence of particle size and extent of demineralization of	In vitro and in vivo (animal study). Dentin-derived granules were chemically treated	Extracted human teeth were milled and divided into 3 groups according to particle size:	micro-CT imaging histomorphometry and immunohistochemical analyses	Completely demineralized dentin matrix granules showed a larger absortion when compared to non-

	dentin matrix on bone regeneration.	considering different protocols resulting in: Completely or partially demineralized dentin matrix. Non-demineralized dentin matrix was also assessed. Granules were implanted into rat calvaria bone defects.	200, 500, and 1000 μm.		demineralized dentin matrix granules. Partially demineralized dentin matrix granules at 1000 μm stimulated a higher proliferation of osteogenic when compared with the other types of granules.

5. Discussion

The present study consists in an integrative review on the effect of dentin matrix graft material used as an alternative bone substitute. Results revealed partially porous granules of demineralized dentin matrix containing hydroxyapatite, opened dentin tubules open and proteins. The bioactive chemical composition and morphological aspects of a partially demineralized dentin matrix induced the proliferation of osteogenic cells and enhanced growth of new bone tissues when compared to non-grafted bone tissues. The findings in literature validate the hypothesis of the present study. A detailed discussion on the tooth-derived dentin graft and its biological effects is described as follow.

5.1 Dentin-derived graft

The chemical composition of dentin and bone are very similar including hydroxyapatite (Hap), type I collagen, and growth factors like insulin-like growth factor (IGF)-II, bone morphogenetic protein (BMP-2), and transforming growth factor (TGF) (12,13). In fact, autologous dentin has inherent biological properties as an autologous bone substitute and shows a low risk of exposing patients to diseases transfer or contaminants. In this way, extracted teeth are source of dentin granules which can be used as bone substitutes for enhanced bone healing (9,11). Teeth are usually extracted due to trauma, advanced periodontal bone loss or other indications like third molars or orthodontic treatment.

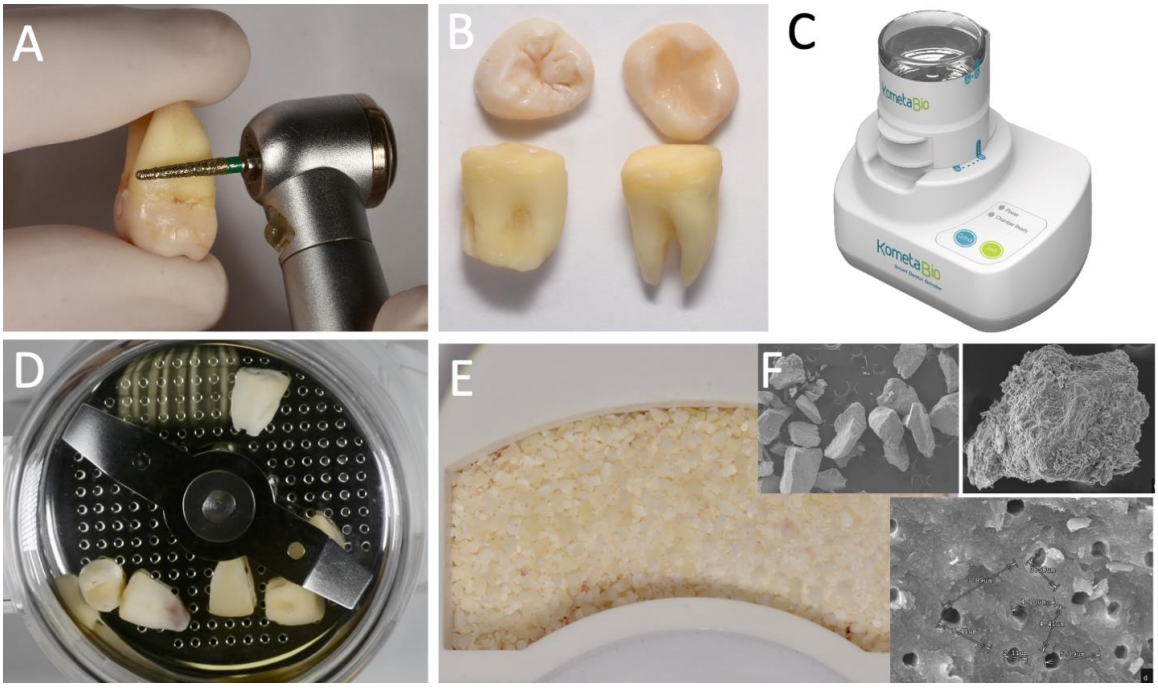

Figure 2. (A and B) Removal of crowns of the extracted teeth. (C) Smart dentin grinder™. (D) Grinding of extracted teeth. (E) Dentin granules. (F) SEM images of the dentin granules.

The preparation of dentin matrix graft is not a standard procedure since several protocols are reported in literature. Recent procedures recommend the removal of enamel and cementum by using tungsten burs to use only dentin from extracted teeth as seen in Figure 2. Immediately after extraction, restorations like crowns or fillings should also be cut off. Tooth roots could be split in case of multi-root teeth. Clean teeth have to be dried by an air syringe and ground in sterile chamber using automatic grinder units such as: Smart Dentin Grinder™, (KometaBio Inc., USA); Bonmaker™ (Korea dental solution, Korea); Mixer Mill M301™ (Retsch GmbH, Germany); Osteo-Mill (Tokyo Iken Co Ltd, Japan), Transformer TT™ (TT Transformer S.r.l, Italy). On the current grinding devices, dentin is planned to be milled into granules with size ranging from 300 up to 1200 µm (Figure 2) (9,11,15). The grinding process is performed at short time (~3s) and then a vibration is performed for 20 s in the collection trays. Sieves are used to separate the granules by size in the range between 300-1200 µm although some adjustment can be performed regarding the sieves

to produce smaller or larger granules. Fine granules with size below 250 μm are not considered by some processing protocols for producing particulate dentin considering previous *in vitro* and *in vivo* studies on bone formation (12,14).

After grinding, the granules are often immersed in solutions for disinfection and removal of smear layer over the granules (Figure 2). At first, granules are immersed in NaOH and ethanol for 5 min as reported in previous studies (9,14,15). NaOH has a strong reactive effect for inactivating virus, bacteria, yeasts, fungi, and endotoxins. That is a strong cleansing and disinfecting agent being able to penetrate and remove the biomass in the dentin tubules, exposing the clean surface of mineralized dentin matrix. As a second step, granules are immersed in EDTA that is a chelating agent known to dissolve hydroxyapatite by removing calcium, thus exposing the organic collagenous matrix and partially demineralizing the dentin granules. The complete removal of smear layer and partially remove of mineral phase of the granules expose the collagen fibers' network and increase the release of proteins like growth factors (12). EDTA also remove Gram-positive and Gram-negative bacteria by binding to Mg^{2+} and Ca^{2+} ions from the bacteria outer cell wall. That leads an inhibition of bacteria adhesion and accumulation as a biofilm. Dentin granules treated with chemical agents possess rough surfaces and open dentin tubules that increase the surface area for interaction with proteins and osteogenic cells when placed in surgical sites (Figure 2).

After immersing in EDTA, dentin granules can be rinsed in sterile phosphate buffered saline (PBS) and then dried at room temperature until clinical use. The time from tooth extraction until grafting takes approximately 15-20 min (9,14,15). Dentin granules can also be chemically treated by other different substances such as HNO_3 or HCl and demineralization depends on the chemical substance and time of immersion (9,14,15). Also, granules can be submitted to a thermal treatment for removal of bacteria and proteins although that also remove the growth factors for bone healing (12). In fact, several protocols for preparation of dentin matrix granules are reported in literature. As seen in Table 1, several other terms have been used for tooth-derived graft materials 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), partially mineralized dentin matrix (PDM) (9,12,14).

5.2 Biological effects

The dentin matrix granules show a rough and porous structure after the mechanically grinding and chemically treatment (16–18). That morphological aspect and chemical composition increases the adhesion, proliferation, and differentiation of osteogenic cells as reported by previous studies (12,13). The stimuli of osteogenic cells promote the deposition of collagen matrix and adsorption calcium and phosphorous and then enhancing the process of mineralization and bone formation.

A previous study evaluated the effects of demineralization on the physicochemical and biological behavior of dentin and enamel particles (13). Teeth were milled sing a Tooth Transformer TT (TT Transformer S.r.l, Italy) producing granules (< 1 mm). Granules were chemically treated with several chemical reagents and then rinsed in distilled water. Cell culture was performed in contact with MG-63 and SAOS-2 osteogenic cells for 3 and 7 days and then the cell viability was carried out using Alamar blue colorimetric assays. Granules showed the presence of collagen and BMP-2. Also, a high viability of osteogenic cells was recorded (13). Another in vitro previous study assessed a deproteinized human demineralized tooth matrix (dDTM) via three methods: (i) thermal treatment, (ii) immersion in NaOH, or (iii) distilled water and then thermal treatment (12). Tooth was milled in granules using a mixer ball mill machine (Mixer Mill M301™, Retsch GmbH, Germany) and then granules were separated usin sieves with 500 µm and 1,000 µm aperture. Hydroxyapatite was detected by XRD analyses after a partially demineralization of tooth granules was performed in 0.5M HCl for 3 h at 25 °C. Cell culture was carried out in contact with MC3T3-E1 osteogenic cells for 1,3,5,7 and 15 days and revealed a high proliferation of cells over dDTM surfaces after treatment in NaOH and thermal treatment (12).

Regarding studies in animals, a previous study evaluated the influence of particle size and extent of demineralization of dentin matrix on bone healing (14). Dentin-derived granules were chemically treated considering different protocols resulting in: Completely (CDDM) or partially demineralized dentin matrix (PDDM). Non-demineralized dentin matrix

(MDM) was also assessed. Granules with different size (200, 500, and 1,000 μm) were implanted into rat calvaria bone defects and micro-CT and histomorphometry assays were carried out. PDDM granules at 1,000 μm stimulated the highest proliferation of osteogenic when compared with CDDM and MDM granules (14). Another in vivo study evaluated the effects of Gamma radiation on the osteoinductive properties of demineralized dentin matrix at extra-skeletal sites (34). Extracted teeth were processed at Korean Tooth Bank (Seoul, Korea) producing dentin granules with a size at 300–800 μm. Dentin granules were ultrasonically cleaned in distilled water and then dehydrated with ethyl alcohol and defatted using ethyl ether solution. Granules were then demineralized for 30 min in 0.6 N HCl. The demineralized particles were lyophilized, packed, and sterilized with ethylene oxide gas. Samples were analyzed by alkaline phosphatase (ALP) and histology on tartrate-resistant acid phosphatase (TRAP) staining. New bone formation was notced in all the groups at each time point. Gamma radiation at doses of 15 and 25 kGy did not affect the osteoinductive capabilities of demineralized dentin matrix (34).

A study in human participants evaluated the efficacy of an autologous dentin graft in preventing periodontal defects after impacted or semi-impacted lower third molars surgical extraction (15). Tooth was milled for producing dentin granules with size range at 300-1,200 μm and then immersed in a 0.5 M NaOH and 20% ethanol solution followed by rinsin in PBS. Extraction sockets from 10 human participants (split-mouth) were filled with autologous dentin graft, while the control sites were filled with blood clot. Post-extractive sites were monitored for 15, 90 and 180 days. Periapical X-ray measurements showed a reduction of the probing pocket depth distal to the second lower molar after six months. X-Ray evaluation also showed a higher amount of bone gain at the grafted sites when compared to the control sites (15). Another study in human participants evaluated the primary stability of delayed implants placed in post-extraction ridges preserved with autogenous mineralized dentin matrix (MDM) versus xenograft granules (9). After 6 months, trephine cores were harvested for histomorphometry prior to implant placement. Implants were then placed, and implant stability was measured immediately as well as two months after placement. Marginal bone loss and presence of mucositis/peri-implantitis were recorded up to 18 months after prosthetic loading. Extracted teeth roots were milled using a Smart Dentin Grinder™, (KometaBio Inc., USA) producing dentin granules with size

at 250-1,200 μm. Dentin granules were immersed in 0.5 M NaOH and 30% (v/v) alcohol for 5 min followed by rinsing in PBS and then dried at room temperature. Clinical, histological and pain experience outcomes were further assessed. Dental implants showed a similar primary (~ 77) and secondary (80-81) implant stability after grafting with MDM granules and xenograft groups. The percentage of newly formed bone around MDM granules (~47%) was significantly higher when compared with xenograft (~35%) ($p < .001$). No significant differences were found as far as clinical, radiographic and patient-related outcomes (9). In another clinical study, radiographical and histological analyses were performed after placement of autogenous tooth-derived graft in the treatment post-extraction sockets with alveolar ridge preservation (28). Enamel-free teeth were milled using using a Bonmaker™ device (Korea dental solution, Korea) resulting in granules with size at 425-1,500 μm. Granules were disinfected for 20min following the manufacturer's instruction. The extraction sockets were filled up with autogenous tooth particulate. Core biopsies showed autogenous tooth particles surrounded by 56% newly bone and connective tissue. Only a mean of 7% of non-remodeled autogenous tooth granule was recorded (28)

6. Conclusion

Within the limitations of the selected studies, the main outcomes of the current integrative review can be drawn:

- Different grinding apparatus are used to manufacturing dentin matrix granules for bone healing. The size of dentin matrix granules ranged from 200 up to 1500 μm although a range between 300 and 1,200 μm was mostly reported in literature. The chemical treatment of granules involved immersion in reactive substances such as NaOH or HCl, or HNO_3 for partially demineralization of the dentin matrix exposing the collagen fibers, opening the dentin tubules' diameter, and releasing growth factors;
- In vitro studies also revealed the presence of hydroxyapatite, type I collagen fibers, and proteins (i.e., BMP-2) in the rough and porous dentin matrix granules. Such chemical composition and rough/porous morphological aspects can provide a high bioactivity and induce the migration and adhesion of proteins and osteogenic cells when placed in bone defects. Cytocompatibility assays revealed a high proliferation and differentiation of osteogenic cells over dentin matrix granules;
- *In vivo* studies revealed a higher amount of new bone around dentin matrix granules in bone defects when compared to non-grafted surgical sites. The amount of new bone was comparable to the sites grafted with demineralized bovine bone mineral. The absorption rate of demineralized dentin matrix granules after implantation was higher when compared to non-demineralized dentin matrix granules. That indicates potential clinical applications in case of early implant placement. Dental implants placed after 6-motnhs bone grafting with dentin matrix granules or demineralized bovine bone mineral revealed similar primary stability.

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