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Review Article

Demineralized dentin matrix for bone regeneration in dentistry: A critical update

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ABSTRACT

Over the last few decades, several new materials and techniques have been developed for bone regeneration. Scaffolds based on demineralized dentin matrix (DDM) present an attractive option due to their availability and several animal and human studies have been conducted to ascertain their utility in regenerative dentistry. The aim of this review was to summarize the recent studies conducted on DDM and used for bone grafts. PubMed, Web of Science, and Scopus were used to search for studies published within the last 10 years. The keywords and terms used were: "demineralized dentine matrix", "bone grafting", "bone augmentation" and "guided tissue regeneration" in various combinations. Original studies (in vitro, animal and human) and systematic reviews were included in the literature search. The literature search initially identified 23 studies (16 animal studies and 7 clinical reports. Most studies included in this review indicate that DDM has demonstrated promising results in a variety of dental and regenerative medicine applications. Further studies are required to completely comprehend its characteristics and prospective applications. Future studies should also focus on optimizing the processing protocols for the production of DDM-based scaffolds.

1. Introduction

The market for dental bone grafts and substitutes has been valued at USD 696.9 million in 2022 and is anticipated to expand at a 9.5 % compound annual growth rate (CAGR) over the following five years (Bohner, 2010). The market for dental bone grafts is expanding as a result of factors, such as increasing periodontal disease incidence, an increasing number of dental implant operations, and an ageing global population. According to the World Health Organization (WHO) nearly 3.5 billion people worldwide are estimated to be affected by oral diseases. In people aged 20 or older, the estimated global average prevalence of complete tooth loss or edentulism is close to 7 % and a substantially greater global frequency of 23 % has been predicted for adults 60 years or older (Felton, 2016; Müller et al., 2007). Losing teeth can be detrimental to an individual's social life, mental health, and is functionally restrictive.

Regenerative dentistry and medicine aim to repair and replace tissues which have been lost due to disease or trauma. There are several major classes of grafts that have been used for bone augmentations and these include natural, synthetic or hybrid materials (Khurshid et al., 2022, 2023; Zhao et al., 2021). Over the last few years, there have been significant advancements in the field of regenerative dentistry, and to induce effective bone repair, a newer materials exhibit a number of key attributes materials (Khurshid et al., 2023; Zhao et al., 2021). *Firstly*, they should maintain a space for the bone and its supporting tissues to proliferate and grow in too. *Secondly*, the graft should enable angiogenesis and regeneration of an extracellular matrix potentially via the development of a blood clot. *Thirdly,* they should be biocompatible and ideally bioactive, capable of stimulating proliferation and differentiation of the cells necessary for repair mechanisms, such as induction of native stem cell processes. Finally, the graft should be degradable and remodel without releasing any cytotoxic or immunogenic substances into the surrounding tissues.

Dentin is the calcified tissue layer of the tooth located beneath the

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outer covering of the enamel (Goldberg et al., 2011). It has a tubular structure with 33 % organic material which consists primarily of collagen, of which 90 % is type 1, as well as 10 % ground substance and proteins, including enzymes, proteoglycans and grown factors (Goldberg et al., 2011). The organic matrix comprises a reservoir of several bioactive and regenerative molecules. These include insulin-like growth factors (IGFs), transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMPs) and basic fibroblast growth factor (bFGF), along with non-collagenous protein (NCPs), such as dentin phosphoprotein (Roberts-Clark & Smith, 2000; Yeomans & Urist, 1967).

Consequently, unlike enamel, it is possible to demineralize dentin and isolate the organic matrix for regenerative applications. This dissolution or demineralization of the dentin is possible due to its decreased total mineral content. Several chemical treatments have been shown to demineralize dentin and these include ethylenediamine tetraacetic acid (EDTA), hydrochloric acid (HCl) and nitric acid (Miller et al., 2021). The method of demineralization, including chemicals used and duration of treatment, impacts the structure of the organic matrix which remains, and this affects the regenerative potential of the demineralized dentin matrix (DDM) generated. Consequently, DDM is a naturally derived biomaterial comprising of a collagen-rich matrix, proteins, lipids and growth factors. Notably, the DDM shares similarities with the bone matrix and is abundant in bioactive molecules, including basic fibroblast growth factor, insulin-like growth factor, transforming growth factor-β, and bone morphogenetic proteins. Yeomans and Urist reported the first application of DDM for tissue regeneration in 1967, and that BMPs from DDM stimulated the formation of new bone (Yeomans & Urist, 1967). Following many subsequent studies DDM has been considered for use as a bone graft material.

The therapeutic efficacy of DDM in regenerative dentistry is understood to be a result of the growth factors which promote the formation of new mineralised tissues including bone and dentin. Furthermore, dentin is an acellular dense matrix with a tubular structure which provides an excellent natural scaffold for bone regeneration. Consequently, animal and human sourced DDM have been studied for potential application as a graft material for various periodontal and maxillofacial bone regenerative applications, such as reconstruction of post-extraction sockets, bone augmentation and guided tissue regeneration (Grawish et al., 2022; J.-K. Ku et al., 2022). The aims of this review are to summarize the major developments and research milestones using DDM for bone regeneration, provide a critical appraisal of the studies conducted in the past decade, the range of chemical treatments used for dentin demineralization and propose future directions for research in this area. Fig. 1 illustrates the sources, processes and steps used to extract DDM for clinical application.

2. Purpose and review methodology

A literature search was conducted to identify published literature which described the potential of DDM for bone regeneration. The following databases: PubMed, ISI Web of Science, and Scopus were electronically trawled for papers published within the last ten years (March 2013 until March 2023). The keywords and terms used were: “demineralized dentin matrix”, “bone grafting”, “bone augmentation” and “guided tissue regeneration” in various combinations. Original studies (in vitro, animal and human) and systematic reviews were included in the literature search. Case reports were also included as they provide information on real-world evidence as they contribute to reporting of rare studies, they play an important role in generating a hypothesis and may also report patient-reported outcomes of clinical procedures. Data were extracted from the publications and presented in tables for synthesis.

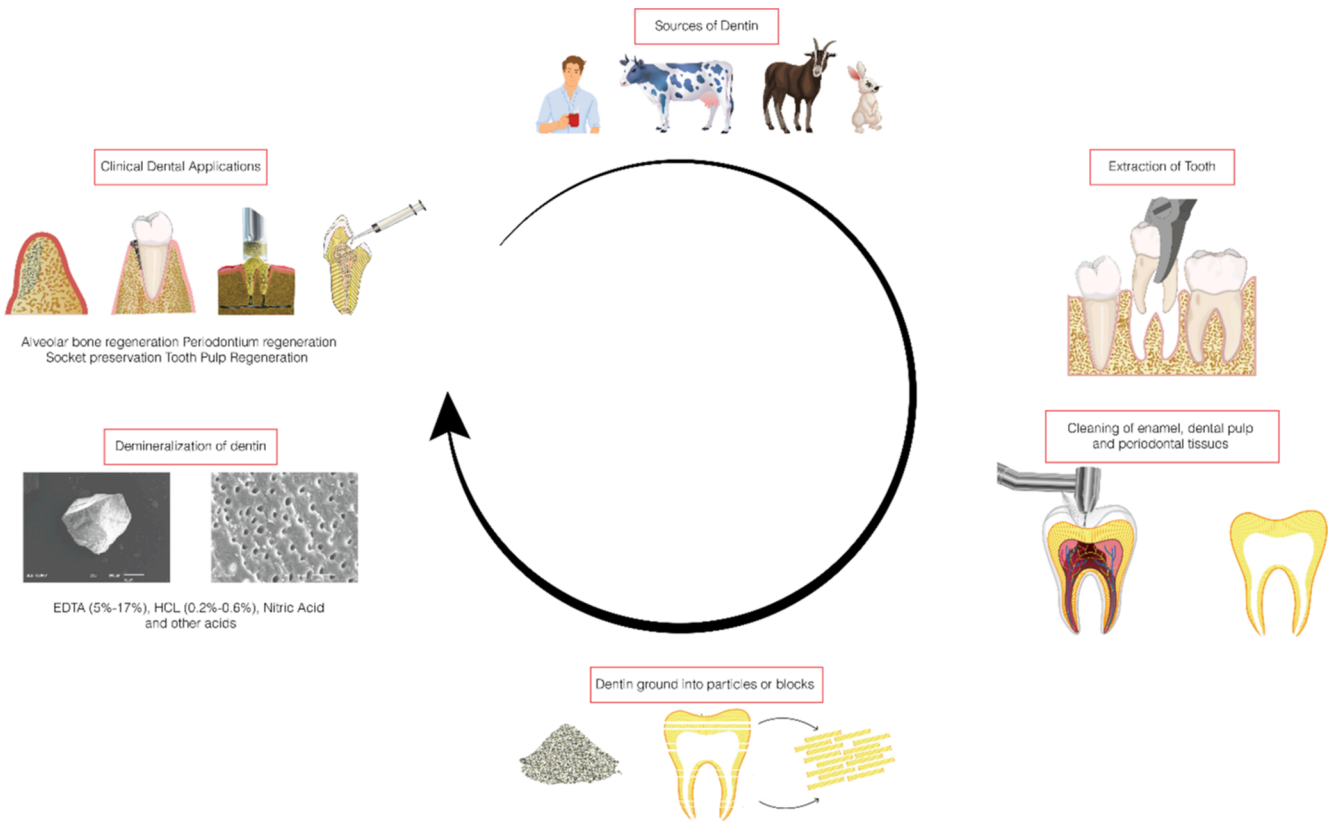

Fig. 1. Processes and steps used to prepare demineralized dentin matrix (DDM) for clinical applications starting with the different sources used for tissue harvesting.

3. Results and discussion

3.1. Results

3.1.1. Search results and general characteristics of studies

The literature search initially identified 23 studies (16 animal studies (Copelli et al., 2021; De Oliveira et al., 2013; Elkady et al., 2023; Fernandes et al., 2020; Gomes et al., 2016; Kabir et al., 2017; B. J. Kim et al., 2021; Kim et al., 2017; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Nam et al., 2016; Qin et al., 2015; Tanoue et al., 2018; Um et al., 2016; Zhu et al., 2021) and 7 clinical reports (Kanazirski & Kanazirska, 2022; Kim et al., 2022; Kim et al., 2016; Minamizato et al., 2018; Murata et al., 2022; Pang et al., 2017; Umebayashi et al., 2020). These are summarized and critically appraised below in Tables 1 and 2. Eight studies used rodents for testing the efficacy of DDM for bone regeneration (Copelli et al., 2021; De Oliveira et al., 2013; Fernandes et al., 2020; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Tanoue et al., 2018; Zhu et al., 2021) and in five studies rabbits were used (Elkady et al., 2023; Gomes et al., 2016; B. J. Kim et al., 2021; Nam et al., 2016; Um et al., 2016), dogs were used in two studies (Kim et al., 2017; Qin et al., 2015) and sheep were used in one study (Kabir et al., 2017).

Table 1
Summary of animal studies conducted for the use of demineralized dentin matrix for bone repair during the last decade.

Study	Animal Model	Demineralizing Agent	Procedure	Experimental/ Control Groups	Study Period	Reported Outcomes
De Oliveira et al., 2013	Rats ($n = 16$)	EDTA	Extraction socket preservation	1. hDDM No treatment	14 days	Increased staining for BMP-2 and BMP-4 in osteoblasts in DDM group.
Qin et al., 2015	Beagle dogs ($n = 6$)	HCl	Calvaria defect fil	1. No treatment Collagen hDDM	3 months	Higher periodontal regeneration observed with hDDM.
Gomes et al., 2015	Rabbits (diabetic/ normal; $n = 60$)	Not stated	Cranium defect fill	1. Normal Diabetic Diabetic + PTFE Diabetic + PTFE + PRP Diabetic + PTFE + DDM	90 days	Highest bone quality and quantity with DDM.
Koga et al., 2016	Rat ($n = 100$)	HCl	Calvaria defect fill	1. Non-DDM Partial DDM Completely DDM	8 weeks	Partial DDM with larger particle size induced prominent bone regeneration.
Um et al., 2016	Rabbit ($n = 12$)	HCl	Calvaria defect fill	1. DDM Bovine bone/ rhBMP-2 DDM/rhBMP-2	8 weeks	DDM and ABB/rhBMP-2 groups showed osteoconductive bone formation, while the DDM/ rhBMP-2 group showed osteoconductive and osteoinductive bone formation.
Nam et al., 2016	Rabbits ($n = 9$)	HCl	Cranium defect fill	DDM (0.1–2 mL; 0.25–2 mm particles)	8 weeks	DDM with a space between particles of 200 μm was effective in bone formation
Kabir et al., 2016	Sheep ($n = 6$)	HNO_3	Iliac crest defect fill	hDDM	4 months	DDM induced bone regeneration.
Kim et al., 2017	Beagle dogs ($n = 8$)	HCl	Extraction socket preservation	hDDM/rhBMP-2	12 weeks	Autogenous bone showed 75 % new bone formation and DDM fixed with rhBMP-2 showed 48 % new bone formation.
Tanoue et al., 2018	Rats ($n = 1$)	HNO_3	Calvaria defect fill	hDDM	12 weeks	Osteocytes of the new bone tissue surrounding the DDM formed a network connected by their cellular processes and formed bone tissue.
Fernandes et al., 2020	Rats ($n = 24$)	EDTA	Extraction socket preservation	1. Blood clot hDDM	21 days	Higher number of trabeculae with hDDM.
Kim et al., 2022	Rabbits ($n = 12$)	H_2O_2	Calvaria defect fill	1. hDDM/PRP hDDM/rhBPM-2 DDM No treatment	8 weeks	The DDM/rhBMP-2 group demonstrated a higher degree of new bone formation. formation and calcification, and the lamellae of bone matrix.
Copelli et al., 2021	Rats ($n = 40$)	EDTA	Subcutaneous replantation	1. Lyophilized DDM MTA Biodentine Empty	30 days	LDDM exhibited similar inflammatory response to MTA and biodentine.
Zhu et al., 2021	Rats ($n = 20$)	NHO_3	Scratched/un-scratched skulls (defect fill)	1. Partial DDM Complete DDM	12 weeks	Completely DDM induced higher bone regeneration.
Ku et al., 2022	Nude mice ($n = 20$)	HCl	Muscles	1. 15 kGy Gamma + DDM 25 kGy Gamma + DDM DDM + no radiation	4 weeks	Gamma radiation did not impact osteoconductivity of DDM.
Moraes et al., 2022	Rats ($n = 1$)	EDTA	Extraction socket preservation	1. Blood clot Autogenous bone Bovine bone hDDM	28 days	DDM induced bone regeneration extraction sockets. No difference between groups.
Elkady et al., 2023	Rabbits ($n = 50$)	EDTA	Mandibular defect fill	1. Rabbit Non-DDM Rabbit DDM + non-DDM No treatment	6 weeks	Hybrid DDM resulted in increased bone regeneration.

Ethylenediaminetetraacetic acid (EDTA), human-derived Demineralized Dentin Matrix (hDDM), Lyophilized DDM (LDDM), Hydrogen chloride (HCL), Polytetrafluoroethylene (PTFE), Platelet-rich plasma (PRP), Recombinant human bone morphogenetic protein-2 (rhBMP-2), Mineral trioxide aggregate (MTA) and Nitric acid (NHO_3).

Table 2
Summary of human clinical studies conducted for the use of demineralised dentin matrix for bone repair during the last decade.

Study	Study Design	Participants	Procedure	Study Group(s)	Follow-Up	Clinical/ Radiographical Assessment	Reported Outcomes
Kim et al., 2016	Case series	N = 5	GBR + Implant placement	ADDM (*n* = 5)	5 years	CBCT, radiography	Successful maintenance of *peri*-implant bone.
Pang et al., 2016	Randomised Clinical Trial	N = 24	Post-extraction bone augmentation	ADDM (*n* = 21 sites) Bio-Oss (*n* = 12 sites)	6 months	Implant stability, histological assessment, vertical bone gain	Similar efficacy in both groups. No difference in listed outcomes between ADDM and Bio-Oss groups.
Minamizato et al., 2016	Cohort	N = 16	Peri-implant grafting, socket preservation, sinus floor augmentation, sinus floor augmentation	APDDM (*n* = 16)	2 years	Radiography, biopsy	Bone regeneration observed in majority of the subjects.
Umebayashi et al., 2020	Case report	N = 1	Anterior maxillary alveolar bone and bilateral sinus floor augmentation	APDDM + PCBM	4 years	CBCT	Stable bone volume.
Kanazirski et al., 2022	Case series	N = 3	Bone augmentation	DDM	3–6 months	CBCT, biopsy	Successful bone augmentation in all patients.
Kim et al., 2023	Retrospective cohort	N = 20	Socket preservation	Immediate DDM Delayed DDM Collagen + bone	2.6–8.6 months	Radiography; bone fill	DDM resulted in higher bone fill than graft alone.
Murata et al., 2022	Case report	N = 1	Autotransplantation	APDDM	18 months	Radiography	PDL space and alveolar ridge observed.

Guided Bone Regeneration (GBR), Autogenous dentin demineralized matrix (ADDM), Cone-beam computed tomography systems (CBCT), autogenous partially demineralized dentin matrix (APDDM) and particulate cancellous bone and marrow (PCBM).

3.1.2. *Demineralizing agents*

For the chemical processing, EDTA was used in five studies as the demineralization agent (Copelli et al., 2021; De Oliveira et al., 2013; Elkady et al., 2023; Fernandes et al., 2020; Moraes et al., 2022) and HCl was used in six studies (Kim et al., 2017; Koga et al., 2016; J. K. Ku et al., 2022; Nam et al., 2016; Qin et al., 2015; Um et al., 2016). In three studies, nitric acid was used (Kabir et al., 2017; Tanoue et al., 2018; Zhu et al., 2021). And, in one study, the processing agent was not stated (Gomes et al., 2016).

3.1.3. *Animal studies*

For clinical translation, in four studies, the DDM was used for socket preservation (De Oliveira et al., 2013; Fernandes et al., 2020; Kim et al., 2017; J. K. Ku et al., 2022) and in 12 studies DDM was used to fill bone defects at various sites within the animal model (Copelli et al., 2021; Elkady et al., 2023; Fernandes et al., 2020; Gomes et al., 2016; Kabir et al., 2017; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Nam et al., 2016; Qin et al., 2015; Um et al., 2016; Zhu et al., 2021). Human DDM was used in four studies (De Oliveira et al., 2013; Kabir et al., 2017; Kim et al., 2017; Qin et al., 2015) and in the other 12 studies, autogenous DDM derived from the extracted teeth of the animals were used for the analyses (Copelli et al., 2021; Elkady et al., 2023; Fernandes et al., 2020; Gomes et al., 2016; B. J. Kim et al., 2021; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Nam et al., 2016; Tanoue et al., 2018; Um et al., 2016; Zhu et al., 2021). The duration of the study periods ranged from 14 days to 4 months (Copelli et al., 2021; De Oliveira et al., 2013; Elkady et al., 2023; Fernandes et al., 2020; Gomes et al., 2016; Kabir et al., 2017; B. J. Kim et al., 2021; Kim et al., 2017; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Nam et al., 2016; Qin et al., 2015; Tanoue et al., 2018; Um et al., 2016; Zhu et al., 2021). In all studies uneventful healing of surgical/implant sites were described (Copelli et al., 2021; De Oliveira et al., 2013; Elkady et al., 2023; Fernandes et al., 2020; Gomes et al., 2016; Kabir et al., 2017; B. J. Kim et al., 2021; Kim et al., 2017; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Nam et al., 2016; Qin et al., 2015; Tanoue et al., 2018; Um et al., 2016; Zhu et al., 2021). In the majority of studies DDM application resulted in higher regeneration of bone compared with control groups (Copelli et al., 2021; De Oliveira et al., 2013; Elkady et al., 2023; Fernandes et al., 2020; Gomes et al., 2016; Kabir et al., 2017; B. J. Kim et al., 2021; Kim et al., 2017; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Nam et al., 2016; Qin et al., 2015; Tanoue et al., 2018; Um et al., 2016; Zhu et al., 2021).

3.1.4. *Human studies*

Four of the clinical studies were case reports or case series (Kanazirski & Kanazirska, 2022; Kim et al., 2016; Murata et al., 2022; Umebayashi et al., 2020). One study reported on a randomized clinical trial (Pang et al., 2017), one study was of a prospective cohort (Minamizato et al., 2018) and another was a retrospective cohort study (Kim et al., 2022). The number of patients treated ranged from 1 to 24, and these were followed up for between 3 months and 5 years.

Kim et al., 2016 and Kanazirski et al., 2022 both described uneventful healing and bon regeneration using DDM (Kanazirski & Kanazirska, 2022; Kim et al., 2016). However, Kim et al. also described placement dental implant concurrently with GBR (Kim et al., 2016). Kim et al., 2023 and Pang et al., 2016 both described similar outcomes following placement of DDM in extraction sockets for socket preservation (Kim et al., 2022) and (Pang et al., 2017). Meanwhile, two studies described using DDM for sinus floor augmentation (Minamizato et al., 2018; Umebayashi et al., 2020). In one study, DDM was used to support autotransplantation (Murata et al., 2022). No complications were reported in any study and all reported favorable clinical outcomes after application of autogenous DDM as a bone augmentation material (Kanazirski & Kanazirska, 2022; Kim et al., 2022; Kim et al., 2016; Minamizato et al., 2018; Murata et al., 2022; Pang et al., 2017; Umebayashi et al., 2020).

3.2. *Discussion*

3.2.1. *Clinical indications of DDM*

In oral and maxillofacial surgery (e.g., dental implantology, sinus graft, cyst defect, cleft alveolus) demineralized dentin matrix has been utilized in bone regeneration procedures (Minetti et al., 2020). The organic matrix of demineralized dentin contains growth factors and collagen, which stimulate osteoblastic activity and promote bone formation. Surgeons often incorporate DDM into grafting materials to

augment bone volume in cases of jaw defects, ridge augmentation, or sinus lifts (Trombelli and Farina, 2008). This approach supports the integration of dental implants and enhances the overall success of implant procedures (Grawish et al., 2022). Additionally, demineralized dentin matrix has been explored for its potential in periodontal regeneration. The growth factors present in DDM have been shown to promote the proliferation and differentiation of periodontal ligament cells and cementoblasts, contributing to the regeneration of periodontal tissues. This application holds promise for the treatment of periodontal defects and the restoration of supporting structures around teeth (Moraes et al., 2022). Indeed, other fields have also seen DDM as an adjunctive treatment modality such as spine surgery (Geiger et al., 2003).

In the realm of tissue engineering, demineralized dentin matrix serves as a valuable scaffold material. Its natural composition and biological properties make it suitable for supporting the growth and differentiation of various cells, including those involved in pulp tissue regeneration. Researchers are investigating the potential of DDM in pulp capping procedures and the development of bioactive materials for endodontic applications. Results of the studies included in this review indicate that DDM provides an effective bone augmentation and socket preservation material. It is postulated that DDM promotes regeneration of tissues and blood vessels due to the presence of its collagenous nanofibrous network (Gupte & Ma, 2012). Notably, hydroxyapatite, the primary mineral component in both, dentine and bone, has also been previously shown to function as an effective bone augmentation material (Sadeghi et al., 2017) An advantage for the use of dentin over bone as a graft material is the presence of a higher collagen proportion (90 %), as this is demonstrated to enhance cell attachment and adhesion (Moussa & Aparicio, 2019). A recent systematic review of clinical trials has demonstrated that autologous DDM is capable of promoting formation of new bone that is comparable to commercial bovine mineralized bone grafts, such as a Bio-Oss® (Li et al., 2022). Notably, autogenous DDM has been observed to degrade more rapidly than Bio-Oss® and has been proposed to promote a higher rate of bone modelling (Li et al., 2022). However, none of the recent studies have been followed up for more than 5 years, which means the long-term efficacy of DDM is unknown.

The majority of recent animal studies reviewed here described the use of an autogenic DDM. Despite it being established that there is a significant increase in the severity of host immune reaction with increasing genetic difference (Keane & Badylak, 2015) [46], even in the studies that used human-derived DDM, successful bone augmentation was observed in animal recipient studies (Copelli et al., 2021; De Oliveira et al., 2013; Elkady et al., 2023; Fernandes et al., 2020; Gomes et al., 2016; Kabir et al., 2017; B. J. Kim et al., 2021; Kim et al., 2017; Koga et al., 2016; J. K. Ku et al., 2022; Moraes et al., 2022; Nam et al., 2016; Qin et al., 2015; Tanoue et al., 2018; Um et al., 2016; Zhu et al., 2021). Conversely, these data suggest that animal sourced DDM may also therefore be an attractive material for bone augmentation in man. Several types of chemicals have been reportedly used for demineralization of DDM. However, to date, no animal or human studies have been performed which compare the demineralizing agents used for its production. *In vitro* elemental analysis of bovine DDM compared with bovine bone-derived matrix revealed that the DDM contains chlorine and calcium in higher concentrations, and this may explain the difference in the osteoconductivity reported between the materials (Mulyawan et al., 2022). Furthermore, it has been proposed that due to the difference between the molecular composition and development of dentin and bone, the extracellular matrices and connective tissue derived from both of these tissues would not only be different but would exert different effects at the implantation site (J. Kim et al., 2021). Notably, HCl at a concentration of at least 0.6 M resulted in almost complete demineralization of dentin whilst maintaining the nanofibrous collagenous matrix (Kim et al., 2014). However, thus far, no recent animal or human study identified conducted investigation into the difference between the biological properties or microscopic topography of these nanostructures. Furthermore, apart from one study, no recent work identified here compared the most efficient method to disinfect dentin which does not compromise the integrity of the collagen matrix.

3.2.2. *Treatment methods of DDM*

A range of concentrations of EDTA (10–17 %) have been used for the demineralization of dentin [18]. A recent study postulated that increasing the time (up to 60 min) for demineralization using 12 % EDTA led to a more uniform and intact tubular structure in the DDM with no significant chemical degradation. Consequently, this material was able to act as an effective scaffold for bone ingrowth (Memè et al., 2022). These data indicate that EDTA is an effective demineralization agent for the production of DDM-based scaffolds and enables no loss of structural integrity. While EDTA is a unique demineralizing agent with the potentiality of chelating the dentin, 'stronger' acids such as HCl have been observed to produce a more degraded and detached tubular structure even when applied at lower concentrations (Kuntze et al., 2020). To date, there is a lack of studies regarding the impact of different demineralization agents on dentin. Investigating and comparing the effectiveness of the different acids on demineralization and the impact of these acids on the structural integrity of the collagenous network and bioactivity of DDM should be the focus of future studies.

3.2.3. *Current scope of DDM in regenerative dentistry*

The outcomes from the clinical studies on the use of DDM provide promising data highlighting its potential as a valuable material for use in a range of dental procedures (Kabir et al., 2017; Kim et al., 2017). Studies, such as the one reported by Qin et al., 2016 (Qin et al., 2015) indicated that DDM can be used effectively in the clinics without any major adverse effects. The socket preservation study by Kim et al., 2023 (Kim et al., 2016), showed that DDM resulted in a higher bone fill compared with a graft alone. This finding indicated that DDM can effectively promote bone regeneration and preserve the socket dimensions following tooth extraction. Socket preservation techniques are crucial in preventing bone loss and maintaining favourable conditions for future dental implant placement. The superior bone fill achieved with DDM highlights its potential as an effective material in socket preservation procedures.

Notably, the absence of complications reported in the studies identified is a significant finding. None of the studies reported any complications related to the use of DDM. These findings suggested that DDM is a safe and well-tolerated material for bone regeneration procedures. The lack of complications supports the feasibility and reliability of incorporating DDM into clinical practice. However, it is important to note that the studies included in the discussion are a combination of animal studies, case reports, case series, cohort studies, and retrospective cohort analyses. While case reports and series provide valuable individual-level insights, they have limitations in terms of generalisability due to their small sample sizes. Cohort studies and retrospective cohorts provide a larger participant pool and comparative analyses, providing more robust evidence.

3.2.4. *DDM as a carrier for growth factors*

In 2007, regulatory approval was granted for the human use of recombinant human bone morphogenetic protein-2 (rhBMP-2) at a concentration of 1.5 mg/mL. This approval included its application with absorbable collagen sponges as an alternative to autogenous bone grafts for procedures such as alveolar ridge augmentation, addressing defects related to extraction sockets, and sinus augmentation. Nevertheless, concerns have arisen due to the use of doses beyond physiological levels and the inadequate retention of rhBMP-2 when delivered via a collagen sponge, leading to dose-dependent side effects associated with off-label usage.

Since 1998, demineralized dentin matrix (DDM), recognized as an osteoinducing bone substrate, has been employed as a carrier for rhBMP-2. Notably, DDM possesses both microparticle and nanoparticle

structures that differ from bone by not undergoing remodeling (Um, 2018). A recent review by In-Woong et al has indicated that in clinical, animal and laboratory studies, DDM can be used to deliver rh-BMP-2 to periodontal tissues in a slow and sustained rate to induced bone regeneration (Um et al., 2020). Hence, making rh-BMP-2 an example of a growth factor that can be delivered safely into the local periodontal tissues. Furthermore, not only other growth factors could be delivered by DDM but may also be combined with other treatment modalities like stem cell therapy (J.-K. Ku et al., 2022).

3.2.5. Relationship between DDM particle size and regenerative efficacy

The relationship between particle size and the efficacy of the DDM has been previously investigated (Koga et al., 2016). Smaller particle sizes have been associated with enhanced cellular responses and increased osteoinductive. It is proposed that their increased efficacy is they provide a greater surface area for cellular interactions, facilitating cell adhesion, migration, and proliferation (Goldberg et al., 2011). These cellular activities are crucial for promoting bone regeneration and wound healing. Furthermore, smaller particles are also know to more effectively release bioactive molecules and growth factors that are naturally present within the dentin, such as BMP and TGF-β family members (Wang et al., 2008). These molecules play a pivotal role in stimulating osteogenesis and tissue regeneration. Interestingly, larger particle sizes of DDM may also offer some advantages as they provide structural stability and contribute to the maintenance of the graft volume (Brunello et al., 2022). Larger particles can therefore act as a scaffold, supporting the ingrowth of new bone and promoting its formation within the defect site. They can also enhance the mechanical strength and integrity of the graft, which can be particularly important at load-bearing sites. To date, limited data exists that compares the clinical efficacy of different particle sizes. Therefore, future studies should focus on determining the ideal DDM particle size and composition to enable optimal bone regeneration. Fig. 2 illustrates the bioactivity of demineralized dentin matrix (DDM) which promotes regeneration of bone.

4. Conclusions

DDM presents an attractive option for bone regeneration and extracted socket preservation. Overall, DDM has demonstrated promising results in a variety of dental and regenerative medicine applications. Further studies are required to provide a more comprehensive characterisation and prospective for future therapeutic applications. Studies should also focus on optimizing the processing protocols for the production of DDM-based scaffolds.

CRediT authorship contribution statement

Zohaib Khurshid: Funding acquisition, Visualization, Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Software, Validation, Conceptualization, Methodology. **Necdet Adanir:** Supervision, Writing – original draft, Investigation, Formal analysis, Data curation, Validation, Methodology, Conceptualization. **Jithendra Ratnayake:** Project administration, Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Software, Methodology, Conceptualization, Validation. **George Dias:** Supervision, Writing – review & editing, Investigation, Formal analysis, Data curation, Validation, Conceptualization, Methodology. **Paul R. Cooper:** Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation, Writing – review & editing, Supervision, Project

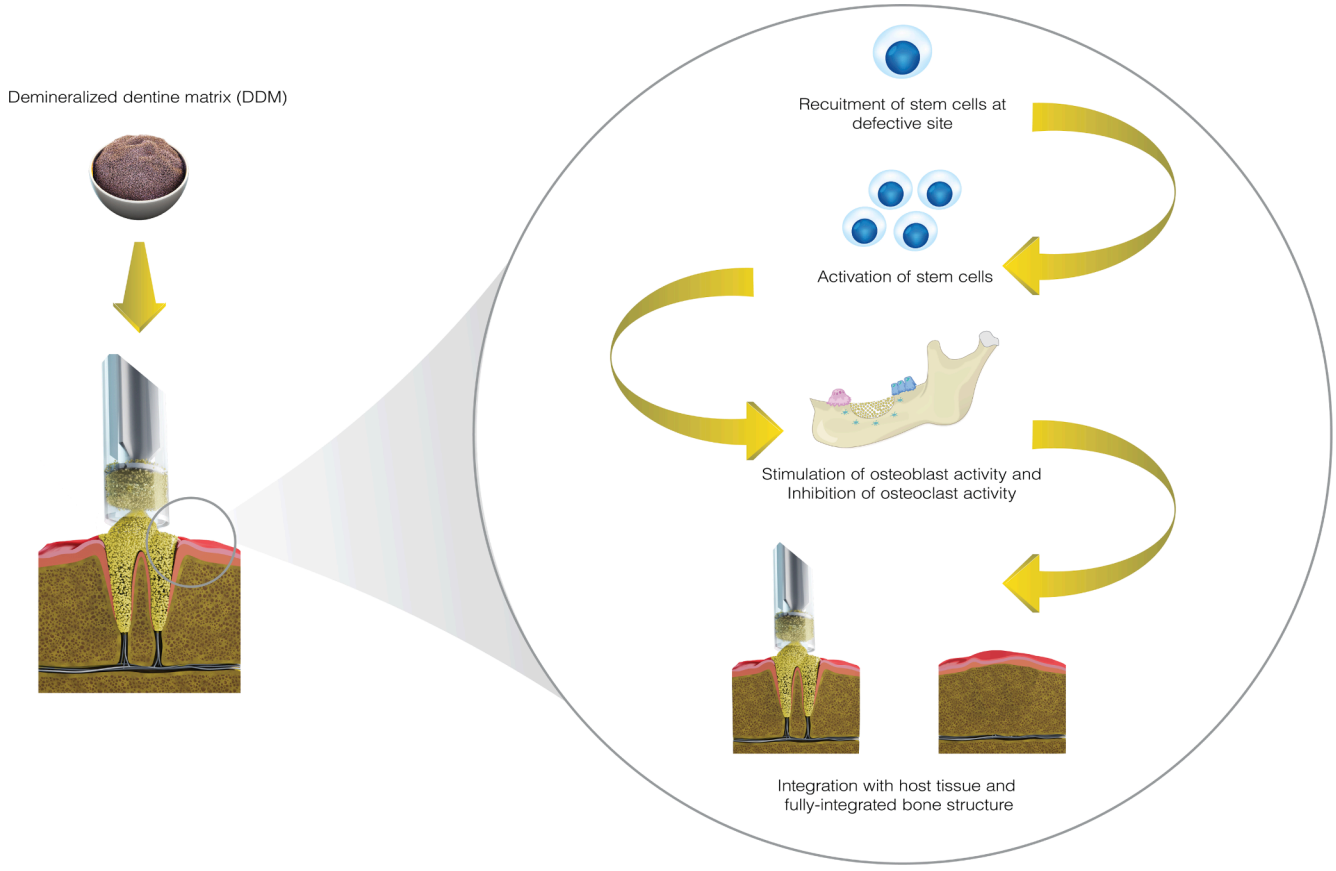

Fig. 2. Illustration showing bioactivity of demineralized dentine matrix (DDM) as a bone graft material. In the example shown, native progenitor stem cells are stimulated by the bioactive molecules from the DDM for attachment, proliferation and differentiate into osteoblasts to enable bone regeneration around a dental implant.

administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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