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Different Methods of Dentin Processing for Application in Bone Tissue engineering: A Systematic Review

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Abstract

Objective: Dentin has become an interesting potential biomaterial for tissue engineering of oral hard tissues. It can be used as a scaffold or as a source of growth factors in bone tissue engineering. Different forms of dentin have been studied for their potential use as bone substitutes. Here we systematically review different methods of dentin preparation and the efficacy of processed dentin in bone tissue engineering.

Methods: An electronic search was carried out in PubMed and Scopus databases for articles published from 2000 to 2016. Studies on dentin preparation for application in bone tissue engineering were selected.

Results: The initial search yielded a total of 1045 articles, of which 37 were finally selected. Review of studies showed that demineralization was the most commonly used dentin preparation process for use in tissue engineering. Dentin extract, dentin particles (tooth ash), freeze-dried dentin, and denatured dentin are others method of dentin preparation.

Conclusion: Based on our literature review, we can conclude that preparation procedure and the size and shape of dentin particles play an important role in its osteoinductive and osteoconductive properties. Standardization of these methods is important to draw a conclusion in this regard.

Key words: Dentin; Biomaterial; Bone substitute; Tissue engineering; Regeneration

Introduction

Despite great advances in dentistry, oral and dental diseases remain a major dilemma worldwide. Oral and dental treatments are often performed using synthetic materials with properties different from those of natural tissues and thus, they eventually fail even under ideal conditions. Tissue engineering is a novel concept in regenerative medicine and dentistry. Using a combination of stem cells, scaffolds and growth factors, tissue engineering offers promising results for regeneration of the injured or lost tissues.[1] Dentin is a suitable biomaterial for use in tissue engineering since it can serve both as a scaffold and a rich source of growth factors. Dentin is a calcified connective tissue and its properties highly depend on its mineralized extra-cellular matrix. This tissue is composed of 50% minerals, 30% organic compounds and 20% water. However, distribution of these components is variable in different parts and different types of dentin.[2, 3] Organic dentin matrix contains macromolecules characteristic of many connective tissues. Also, this matrix contains compounds specific for mineralized tissues.[4] The matrix is synthesized by odontoblasts and is a rich source of growth factors and bioactive molecules required for dentinogenesis, which are released in presence of bacterial acids or some dental materials in case of caries or restorative treatments and cause regeneration and repair of dentin.[5-15] Dentinal matrix compounds released by ethylenediaminetetraacetic acid (EDTA) etchants have shown significant morphogenetic activities and caused induction of dentinogenesis *in vivo*.[16] Dentin organic matrix includes non-collagenous (NCPs) and collagenous proteins, proteoglycans, glycoproteins and lipids. Collagens are the most abundant dentin extracellular matrix proteins (90%), which are mainly composed of type I collagen as in bone. Dentin collagen forms a compact and cross-linked scaffold in which, mineral crystals deposit [17, 18] and contains several growth factors such as transforming growth factor-β (TGF-β1), insulin-like

growth factor (IGF), bone morphogenetic proteins (BMPs) as well as some angiogenic growth factors.[19-21] These growth factors are released secondary to processes such as caries progression, which result in dentin dissolution and stimulate reparative dentinogenesis. [22] The family of TGF-β has been identified in human dentin, pulp cells and odontoblasts and its significant role in regeneration and repair has been well elucidated.[23] The TGF-β1 and BMP-2 can induce differentiation of dental papilla cells to odontoblasts and result in formation of tooth components. [24] *In vitro* and *in vivo* studies on the application of extrinsic growth factors especially TGF-β1 on exposed pulp have shown the pivotal role of these molecules in signaling of odontoblast differentiation in regenerative processes.[25-32] Also, the important role of TGF-β1 in treatment of tooth mineralized matrix defects and extensive oral inflammation in mice has been demonstrated.[33] Several groups of NCPs are available. One group only contains proteins specific to dentin. This group of proteins, expressed by odontoblasts only, is referred to as dentin-specific proteins [34] and includes dentin phosphoprotein (DPP), dentin sialoprotein (DSP) and dentin matrix protein 1 (DMP1). Another group of NCPs in dentin are the extracellular matrix proteins found in bone, dentin and cementum; they are secreted by specific cells in these tissues. This group included bone sialoproteins (BSP), osteocalcin and bone Gla-protein (BGP). This group is recognized as specific proteins of the mineralized tissues.[34] The NCPs play a role in initiation and control of mineralization of collagen fibrils and growth of crystals during dentin formation.[35] Dentin also includes other macromolecules synthesized by odontoblasts or other cells, which are also found in the extracellular matrix of other tissues. This group included osteopontin and osteonectin. [36]

The efficacy of dentin in regeneration of tooth and bone has attracted the attention of researchers in the recent years.[37-39] Due to the high similarity of dentin and bone in terms of their chemical

composition (35% organics and 65% minerals), researchers have considered dentin as an alternative for use in bone regeneration. [40, 41] In this regard, a question arises that whether dentin requires any processing prior to use in tissue engineering and that what techniques have been used for dentin processing so far. On the other hand, many studies have used dentin for regeneration of bone defects. However, its efficacy in comparison with other materials for osteogenic differentiation of stem cells is still a matter of question.

Considering all the above, this study aimed to review different dentin preparation methods and assess the efficacy of modified/unmodified dentin for use in bone tissue engineering.

Materials and Methods

After defining the question of the study, the key words were extracted. PubMed and Scopus databases were searched using the key words: "dentin, tooth, stem cells, osteogenic differentiation, graft, bone regeneration, dentin preparation, processed dentin, tissue engineering, dentin non-collagenous proteins, demineralized dentin, denatured dentin, and tooth ash". Only articles published after January 1st, 2000 in English language with the objective of dentin application in bone tissue engineering were included. Studies on the use of dentin for regeneration of non-osseous tissues such as tooth, cartilage, cementum, etc. were excluded. Articles on dentin preparation with the aim of assessment of its mechanical properties or the effects of bonding agents, etc. were excluded as well. Eventually, 37 studies were selected for final analysis and review.

Results

A. *Dentin preparation methods*

Several protocols have been used for dentin preparation for application in tissue engineering in different studies. These protocols can be divided into four main categories:

1. *Dentin preparation by extraction of NCPs*

The method introduced by Smith et al. may be one of the oldest protocols for extraction of dentin NCPs. Many studies have been published in this regard earlier than 2000. At present, this method with slight modifications is still used as a suitable technique. The flowchart of dentin preparation using this protocol is shown in Figure 1.[42]

Another common protocol for extraction of dentin proteins is to use guanidinium chloride. [43] At present, some other materials such as acids, calcium hydroxide and different types of mineral trioxide aggregate (MTA) are used for extraction of dentin proteins. However, it appears that the use of EDTA results in the highest level of extraction. [44] Table 1 shows different methods used for this purpose so far.

2. *Dentin preparation by demineralization*

The method described by Reddi et al. is among the oldest methods of dentin demineralization, which is still used with some modifications [47]. Since then, some other methods have also been used for dentin demineralization. Table 2 presents a list of these methods.

3. *Dentin preparation by elimination of organic matrix*

Denaturing dentin has been evaluated in a small number of studies. In fact, it seems that due to the significance of dentin matrix proteins in migration, adhesion, proliferation and differentiation of cells, most researchers have attempted to prevent denaturation of dentin

in its preparation process. However, some researchers have evaluated dentin preparation by elimination of its organic matrix. Table 3 presents studies conducted after the year 2000 in this regard. This method has also been used in some other studies; however, they were excluded from our list since they did not use an in vitro method for evaluation of dentin properties.

4. *Dentin preparation and its application without major modifications*

In most studies on different types of prepared or modified dentin, the control group included unprepared or unmodified dentin; however, the control group often underwent some modifications such as lyophilization or nitrogen tank storage. The results of most studies have shown that dentin modification (demineralization or denaturation) improves dentin properties for use in tissue engineering.[34, 21] However, the process of use of dentin that has not undergone modifications such as demineralization or denaturation is particularly important. Studies have shown that storage in liquid nitrogen does not affect the strength while autoclaving can decrease the strength of dentin.[57]

B. Studies on the osteogenic effects of dentin:

1. *Clinical studies*

In 2003, a study evaluated the osteoinductive properties of autogenous demineralized dentin matrix (ADDM) particles for the maxillary sinus augmentation. It was the first report of the successful use of ADDM.[58] Since then, several studies have clinically assessed dentin applications for bone regeneration. Table 4 lists studies in this regard.

2. *In vitro studies:*

In vitro studies on the effect of different forms of dentin on different cells and showing their differentiation to osteoblasts were evaluated. Studies on cytotoxicity or differentiation to

other cell lines were excluded. Although the effects of different forms of dentin on differentiation of stem cells to cementoblasts, chondroblasts and odontoblasts were evaluated, number of in vitro studies on its osteogenic effect was scarce (Table 5).

Discussion

Bone and dentin are hybrid composites composing of organic and inorganic proteins and minerals with high fracture strength, hardness and toughness. Dentin matrix is considered a suitable alternative to bone grafts in reconstruction and regeneration of maxillofacial defects due to having BMPs, which induce and enhance osteogenesis [67] as well as some other optimal properties.

Different forms of dentin have been evaluated in previous studies such as NCPs extracted from dentin, dentin particles (tooth ash), freeze-dried dentin, denatured dentin, freeze-dried and demineralized dentin and demineralized dentin. Lyophilization and nitrogen tank storage are often used to decrease the antigenicity of biomaterials.[80] Thus, these protocols have been used in most previous studies on dentin.

Non-collagenous proteins are extracted from dentin by use of different techniques, some of which have been patented.[81] The optimal efficacy of these proteins for odonto/osteogenic differentiation of stem cells (even those isolated from the endometrium) has been confirmed [82]. Moreover, the effect of these proteins on proliferation of dental pulp stem cells and their synergistic or antagonistic effects in conjunction with factors such as PDGF and TGF have been well demonstrated. Studies have shown that the concentration and pH of the extracting material and duration of exposure are extremely important.[83] Also, in order to prevent protein destruction in the process of extraction, protease inhibitors must be used.[48] Although EDTA has been recognized as an effective material in extraction of these proteins, no study has compared all the available extraction protocols for dentin NCPs.

Demineralized dentin is an organic resorbable material that contains natural growth factors; after placement in the body, it absorbs some of the body fluid proteins.[84] What matters the

most in the process of demineralization is to prevent protein denaturation.[63] It appears that demineralization helps the release of growth factors and proteins and results in osteoinduction.[85] In previous studies, EDTA, phosphoric acid, chloridric acid, nitric acid, hydrogen oxide, ethyl ether and ethyl alcohol have been used for dentin demineralization and scanning electron microscopy (SEM) and X-ray diffraction (XRD) are among the most commonly used modalities to confirm demineralization of samples and exposure of dentinal tubules.

Review of the literature revealed that only a few studies have been conducted on denatured dentin for bone tissue engineering.[56, 76, 55] This may be due to poor mechanical properties of denatured dentin, which do not make it a good candidate for use in stress bearing areas of bone. In such conditions, a combination of dentin and a polymer like chitosan or poly (lactic co-glycolic) acid (PLGA) can be preferably used. Dentin particles (tooth ash), introduced by Kim et al, in 1993, have been used ever since; tooth ash is prepared at high temperatures via a dentin denaturation protocol. Microscopic analyses confirmed that hydroxyapatite and tricalcium phosphate are the main constituents of this powder.[86] In most studies conducted by Kim et al, a combination of dentin powder, Paris plaster and platelet-rich plasma (PRP) has been used.[70] Moharamzadeh et al, in their study used a preparation method similar to dentin denaturation technique.[55] Sodium hypochlorite (5.25% NaOCl for 5 days at 4°C) can also be used for denaturation of dentin;[87] but there was no study using this method in bone tissue engineering. It is particularly important to use scaffolds with properties similar to those of defected tissue.[41] Apatite in bone tissue has low crystallinity with nanometer-scale particle sizes. By an increase in its crystallinity and size of particles, its biodegradation in the human

body will become impossible and its osteoconductivity will decrease.[40] This issue must be taken into account in simulation of natural denaturated scaffolds containing hydroxyapatite.

At present, many clinical studies are ongoing on tooth derivatives instead of autogenous bone for use as graft in bone defects. The first bone autograft was used in human in 1820 while the first dentin autograft was first used in human in 2003.[58] Many researchers have suggested that this material can serve as a carrier for growth factors and stem cells in defects.[47, 22] Studies in this respect led to the development of AutoBT technology (Korea Tooth Bank Co., Seoul, Korea), which uses the extracted teeth of the same individuals; this technique has already gained popularity in Korea and Japan.[54] Although evidence shows appropriate response and better bone regeneration in presence of different types of dentin, a study in 1998 reported that partially demineralized dentin granules did not cause osteoinduction.[88] Controversy exists regarding the ideal size of dentin particles, which has been reported to be 75 to 500μ.[81] Some studies have added plaster or beta tricalcium phosphate to the mixture to homogenize the size of particles. However, some researchers claim that the size of particles should not be necessarily homogenized.[84] Some others have shown that demineralized dentin is more active than calcified dentin. They claim that the process of demineralization increases osteoinductivity and decreases antigenicity.[53, 52] That is probably the reason behind the use of demineralized dentin in animal and human studies after the year 2000. A noteworthy issue is that allogeneic dentin (dentin from different species) has been used in some previous studies with no inflammatory or foreign body reactions.[89]

Review of the literature revealed that only a small number of studies have used bone-derived materials compared to dentin. One previous study reported that the efficacy of different products derived from dentin was lower than the efficacy of those derived from bone for

bone regeneration.[60] Another study reported the same result as well.[73] However, to make a right decision regarding the selection of a biomaterial for bone defects, further studies with simultaneous comparison of bone substitutes must be carried out.

Also, the size of bone defects created in most previous studies was small; but presence of a negative control group can well reveal the speed of bone formation in dentin groups. Furthermore, presence of bone graft as a positive control can show the effect of dentin as a bone graft and superiority of groups.

It should be noted that teeth contain many organic components even after a long storage following extraction because solid apatite present on the surface and in the tooth structure prevents the extrusion of organic materials.[90] Considering the same origin of teeth and the alveolar bone (both derived from the neural crest), use of dentin particularly in alveolar bone defects must be further scrutinized.

Teeth are extracted and discarded every day while they may serve as a suitable biomaterial in near future. The role of dental pulp stem cells has long been elucidated in bone regeneration;[91] however, one issue that must be taken into account is that the high cost of isolation and culture of stem cells and high cost of growth factors are among the main challenges in tissue engineering. Dental biomaterials science can play a pivotal role in decreasing the costs of tissue engineering by designing bio-scaffolds inducing the migration of stem cells and delivering effective factors to the respective area after placement in the tissue. They should be designed in such a way to support different types of cells similar to the extracellular matrix of natural bone.

Conclusion

Production of accessible, bioabsorbable materials triggering no adverse immunity reaction and causing fast regeneration of bone is a challenge in tissue engineering. Based on the reviewed studies, it may be concluded that dentin preparation protocol and size and shape of dentin particles play a pivotal role in osteoinductivity and osteoconductivity of dentin. Finding an efficient and affordable dentin preparation protocol and its comparison with bone grafts is an important step in this regard. Professional cooperation of dental material specialists with engineers and surgeons can help achieve this goal and take a step forward in this way.

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Figure Legends

Figure 1. The flowchart of extraction of dentin NCPs by use of EDTA

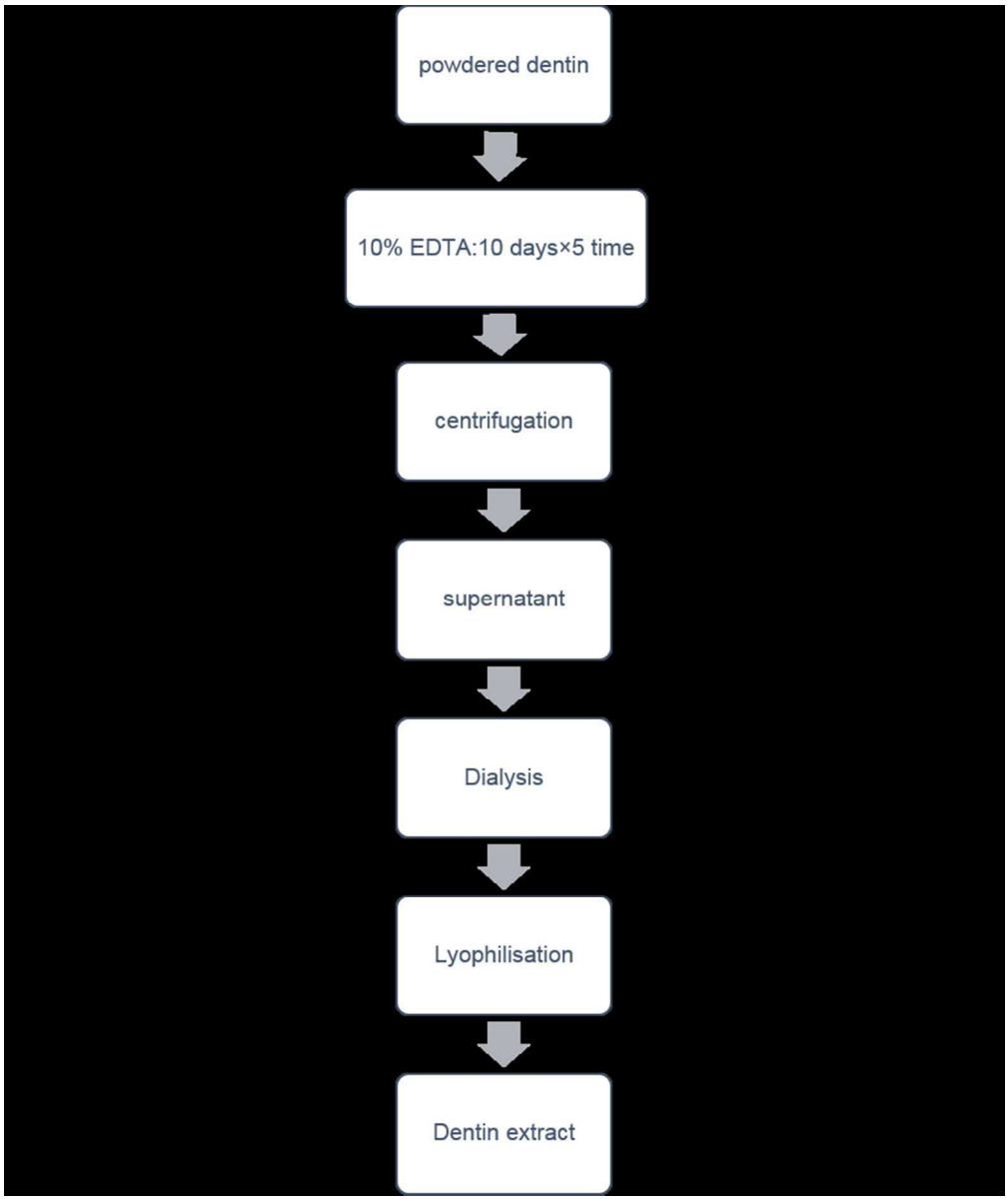

Figure 1. The flowchart of extraction of dentin NCPs by use of EDTA
153x182mm (150 x 150 DPI)

Table 1. List of studies on dentin preparation by extraction of its NCPs

Authors	Publication date	Species	Preparation process	Final product form	Evaluation methods	Results
Martin-De Las Heras et al.[43]	2000	Human	Guanidinium chloride for 4 days+ EDTA for 16 days+ guanidinium chloride for 3 days	Lyophilized	Electrophoresis, Zymography and western blot	Diversity of proteins and identification of active form of gelatinase
Tomson et al.[44]	2005	Human	EDTA, calcium hydroxide, white and gray MTA for 40 days	Lyophilized	Electrophoresis and comparison of compounds with specific kits	The highest amount of extracted protein in EDTA group
Graham et al.[5]	2006	Human	EDTA, calcium hydroxide for 14 days	Lyophilized	Electrophoresis and comparison of compounds with specific kits	The highest amount of extracted protein in EDTA group
Huang et al.[45]	2008	Rat	Guanidium-HCl for 15 hours		Chromatography and western immunoblotting	Difference of proteins in dentin and bone extract
Kim et al.[46]	2009	Guinea pig	EDTA containing protease inhibitor for 14 days		Electrophoresis and western blot	Optimal purity and diversity of proteins

EDTA: ethylenediaminetetraacetic acid, MTA: mineral trioxide aggregate, HCl: hydrochloride

Table 2. Studies on dentin preparation via its demineralization

Authors	Publication date	Species	Preparation process	Final product form	Evaluation methods	Results
Parmar et al. [48]	2004	Human	EDTA with different concentrations and pH values	Root dentin in equal sections	Measurement of released phosphorous by a spectrophotometer	Higher levels of phosphorous were released at the pH of 7.5 compared to 9 and 17% EDTA concentration compared to other concentrations
Vennat et al.[49]	2009	Human	Phosphoric acid, EDTA, hexamethyldisilazane or lyophilization	Disc	EDS X ray, SEM, microanalysis and porosimetry	Higher porosities and lower shrinkage in the freeze-dried group
Yagihashi et al. [50]	2009	Bovine	Demineralization in HCl for one week, chloroform methanol for one day	Lyophilized powder in 250-500µ sizes	Electrophoresis	Detection of BMP in the prepared powder
Chun et al.[51]	2011	Human	10 minutes of ethanol, freeze-drying, use of EDTA or HCl for two weeks	Lyophilized powder in 100-250 µ sizes	FTIR and SEM	Smoother surface of group decalcified in EDTA compared to the rougher surface of group decalcified in HCl. Presence of more favorable superficial groups in the group decalcified in EDTA
Kim et al.[52]	2011	Human	Two groups: freeze-dried tooth, partially decalcified and freeze-dried (AutoBT)	Freeze-dried powder	XRD, EDS and SEM	Higher similarity of AutoBT to bone tissue
Li et al.[39]	2011	Human	Different concentrations of EDTA	Decalcified dentin matrix compared to unmodified dentin and HA/TCP	ELISA and SEM	Exposure of dentinal tubules in the modified group, no difference in the concentration of proteins in the modified dentin and unmodified dentin groups, absence of protein in HA/TCP group
Akazawa et al.[53]	2012	Human	10 to 60 minutes in nitric acid or chloridric acid solutions at different temperatures	DDM granules	Assessment of bioactivity in SBF, XRD, SEM, EPMA and ICP	Elimination of hydroxyapatite crystals within 60 minutes and decrease in weight to 1/50, deposition of hydroxyapatite on granules in SBF, difference in the morphology of deposits depending on the concentration of acids
Kim et al.[54]	2014	Human	Hydrogen oxide, ethyl alcohol, ethyl ether	AutoBT lyophilized powder compared to bone grafts, xenografts, allografts and alloplast	SEM, XRD and CaP solubility test	Surface texture of AutoBT similar to autogenous bone, less crystalline structure compared to autogenous bone and solubility similar to that of autogenous bone

SBF: Simulated body fluid, ICP: Inductively Coupled Plasma Mass Spectrometer, CaP: Calcium phosphate, HA/TCP: Hydroxyapatite/three calcium phosphate, DDM: Demineralized dentin matrix, FTIR: Fourier transform infrared spectroscopy, XRD: X-ray diffraction, SEM: Scanning electron microscope, EPMA: Electron Probe Micro-Analysis

Table 3. List of studies on dentin preparation by its denaturation

Authors	Publication date	Species	Preparation process	Final product form	Evaluation methods	Results
Moharamzadeh et al.[55]	2008	Bovine	Boiling dentin in water for 2 hours, 2 hours in isopropanol, drying at 100°C	Powder	Biocompatibility test with Alamar Blue	Excellent biocompatibility
Elkayar et al.[56]	2013	Bovine	Boiling dentin in water for 90 minutes, calcination in humid environment at 735°C, sintering at 1150°C	Dentin powder in 45 micron size	EDX, IR, XRD, Emmett, Brunauer (BET) and Teller to assess surface topography and thermal stability and SEM	Hydroxyapatite was successfully produced. Presence of hydroxyl and phosphate functional groups was confirmed. SEM showed porous structure of matrix. Constitutional elements of the sample such as calcium, potassium, phosphorous, etc. were identified by EDX.

EDX: energy-dispersive X-ray, IR: Infrared spectroscopy, XRD: X-ray diffraction.

Table 4. In vivo studies using dentin for bone or tooth regeneration

Authors	Publication date	Species	Type of dentin used	Other materials used in combination with dentin	Comparison groups	Defect	Results
Gomes et al, [59]	2001	Rabbit	ADDM	HAM	HAM alone	Surgery in parietal bone	Faster healing in ADDM group
Kim et al.[60]	2001	Rat	Dentin particles	Plaster	Group 1. Dentin and plaster Group 2. Dentin, plaster and Bio-Oss Group 3. Plaster and Bio-Oss Group 4. Bio-Oss alone Group 5. Defects with no treatment	Calvarial defects	New bone formation was the highest in group 4 followed by groups 3, 2, 1 and control
Kim et al.[61]	2002	Dog	Dentin particles	Plaster	Group 1. No treatment Group 2. Plaster and dentin particles Group 3. Plaster, dentin particles and PRP	Round bone defects in the iliac crest	Higher percentage of bone contact in group 3
Gomes et al.[62]	2002	Rabbit	ADDM	PTFE	PTFE alone	Surgery in parietal bone	Faster radiopacity in ADDM group
Murata et al.[58]	2003	Human (first reported human case)	ADDM	PRP and AFDBM	-	Severe atrophy of the posterior maxilla	Radiopacity similar to cortical bone density, osteoinduction
Carvalho et al.[63]	2004	Mouse	HDDM	-	Control group: With bone defects Group 2. Bone defect with PTFE membrane Group 3. Bone defect with PTFE and HDDM	Surgically created defects in the mandible	Higher volume of bone matrix in PTFE+HDDM and HDDM groups
Kim et al.[64]	2004	Rat	Tooth ash	Plaster	Group 1. Surgical removal of ovaries with no graft Group 2. Surgical removal of ovaries and plaster bone powder graft Group 3. No surgery or graft Group 4. No surgery but plaster tooth powder graft	Round bone defects 8mm in diameter in the calvaria	Significantly higher bone formation in tooth powder-plaster group
Murata et al. [65]	2005	Mouse	Human DDM	-	Combination of recombinant BMP2 and DDM	Subcutaneously in the skin	Induction of bone and cartilage, better response of DDM/BMP2
Gomes et al.[66]	2006	Human	ADDM	PTFE	Control group: Tooth socket with no treatment PTFE group: Tooth socket covered with PTFE membrane	Healing of human third molar tooth socket	More homogenous radiopacity, density closer to natural bone, faster healing in ADDM group
Gomes et al.[67]	2007	Rabbit	HDDM	PTFE	Control group: Healthy with no treatment, Diabetic group with no treatment Diabetic group with PTFE	Surgery in parietal bone	Higher density and more organized bone in PTFE+ HDDM group
Park et al. [68]	2008	Rat	Porcine	-	Control group: Healthy with	Calvarial	Highest bone formation in

			dentin particles		no treatment, Porcine dentin particle: group 1, Mixture of dentin particle and Paris plaster: group 2, Dentin particle and chitosan: group 3 Chitosan alone: group 4	defect 8mm in diameter	group 4 followed by groups 2, 3, 1 and control
Gomes et al. [69]	2008	Rabbit	HDDM	PTFE	Control group: Healthy with no treatment Diabetic group with no treatment, Diabetic group with PTFE	Surgery in parietal bone	Higher density and more organized bone structure in PTFE+ HDDM group
Moharamzadeh et al. [55]	2008	Rat	Bovine dentin without organic materials	-		Bone defects in femur	New bone formation and no reaction of the immune system
Yagihashi et al.[50]	2009	Rabbit	Bovine DDM	-	Control group: No treatment, Groups II and III: Different amounts of DDM	Articular cartilage defects	Superior bone regeneration in group III
Kim et al.[70]	2010	Rabbit	Denatured dentin	Plaster	Group 1: Control, Group 2: Tooth powder and Paris plaster, Group 3: Tooth powder- Paris plaster and PRP, Group 4: Tooth powder, Paris plaster and fibrin sealant	Round 8mm calvarial bone defects	Bone formation was higher in groups 3 and 4 than in group 2 but this difference was not significant
Kim et al.[71]	2010	Rat	Denatured dentin	Plaster	Group 1: No graft Group 2: Dentin particle and Paris plaster Group 3: Tisseel, dentin particle and Paris plaster Group 4: Tisseel graft only	8mm calvarial defect	Highest osteogenesis was seen in group 2 followed by groups 3 and 4
Kim et al.[72]	2010	Human	Denatured dentin	-		Jawbone defects	At 6 months, high amounts of the material were eliminated and replaced with trabecular bone
Nampo et al.[73]	2010	Rat	Untreated teeth	-	Control: No treatment, Treatment with bone autograft	Alveolar bone defects	Bone formation in group treated with dentin similar to that in group treated with bone at 8 weeks
Chun et al.[51]	2011	Mice	Human DDPs	PLGA with dental pulp stem cells with bone marrow	Control: PLGA with cells and 4 groups of scaffold containing 1, 3, 5 and 10wt% DDPs	Calvarial defects	Higher bone formation in scaffolds containing 1 and 3wt% DDPs compared to other groups and better response in combination with bone marrow cells compared to pulp cells but with a lesser extent
Togari et al.[74]	2012	Rat	Demineralized dentin	-	Control group	Calvarial defects (parietal bone)	Uniform and continuous bone formation in the graft group
Reis-Filho et al.[75]	2012	Rat	human demineralised dentine matrix (DHDM)	-	Blood clot (control group)	Extracted tooth socket	DHDM increased the expression of VEGF and enhanced the process of tooth socket healing in rats by inducing bone substitution and formation

							of blood vessels
Ibrahim Hussain et al.[76]	2012	Rabbit	processed bovine dentin	-	Control: No filling Group I: Filling the defect with autogenous bone Group 2: Filling the defect with dentin	Round calvarial defects 8mm in diameter	Higher density in areas of dentin grafts compared to the surrounding bone and areas of autogenous bone grafts. Dentin particles were surrounded by capsular soft tissue after 6 weeks
Akazawa et al. [53]	2012	Mouse	demineralized dentin matrix granules	-		Subcutaneously in the back	Bioabsorption along with a few giant cells around the superficial layer of granules
Kim et al.[77]	2013	Human	autogenous tooth blocks	-		Jawbone defects along with implant	In all patients, optimal bone healing was observed but implant osseointegration did not occur in one patient.

ADDM: autogenous demineralized dentin matrix, HAM: human amniotic membrane, PTFE: polytetrafluoroethylene, PRP: platelet-rich plasma, AFDBM: autogenous freeze dried demineralized bone matrix, HDDM: human demineralized dentin matrix, DDM: demineralized dentin matrix, DDP: demineralized dentin powder.

Table 5. In vitro studies using dentin to induce differentiation of cells to osteoblasts

Authors	Publication date	Type of dentin used	Type of cells used	Comparison groups	Evaluation methods	Results
Liu et al.[78]	2005	Dentin extract of guinea pig	Human dental pulp stem cells	Cells subjected to extract with or without osteogenic medium	Real-time PCR and von Kossa staining	Confirmation of differentiation in groups containing extract and osteogenic medium
Chun et al.[51]	2011	Composite scaffold of DDP and PLGA	Human dental pulp stem cells or human bone marrow stem cells	Cells on scaffold or in osteogenic medium	Measurement of alkaline phosphatase, Alizarin staining, RT-PCR	Higher expression of alkaline phosphatase in scaffold+ osteogenic medium group compared to osteogenic medium alone, expression of osteogenic markers
Yu et al.[79]	2014	Dentin NCPs	Bone marrow stem cells	Different ratios of proteins	Measurement of alkaline phosphatase, Alizarin staining, RT-PCR	Increased alkaline phosphatase activity, mineralization and expression of osteogenic markers in presence of 10 mug/mL of dentin proteins

PLGA: poly (lactic co-glycolic) acid, RT-PCR: real‐time polymerase chain reaction, DDP: demineralized dentin particle.