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DEMINERALIZED DENTINE MATRIX AS A CARRIER OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2 FOR HARD TISSUE REGENERATION. A REVIEW OF LITERATURE.

Dental Science

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ABSTRACT

Demineralized dentine matrix (DDM) is a newly developed biomaterial, based on the autogenous tooth dentine and formed through demineralization. Dentine consist of extracellular collagen type 1 and many growth factors due to this it percieves osteoconductive and osteoinductive potenti: DDM is nowadays used in bone regeneration procedures like socket preservation, guided bone regeneration, maxillary sinus lift and also for the carrier of growth factors like rhBMP-2.The rhBMP-2 exhibit bone inducing properties and in combination with DDM, they exhibit synergistic properties. For this review literature search was performed on PubMed, PubMed Central, MEDLINE, Scopus, Google Scholar, and Google electronic search engines and all kind of literature available like original paper, past reviews, case reports were included. This review aims to critically appraise and evaluate the Demineralised Dentine Matrix (DDM) as a novel and reliable scaffold carrier for recombinant human bone morphogenetic protein-2 (rhBMP-2) growth factor.

KEYWORDS

Demineralized Dentine Matrix, DDM/rhBMP-2 preparation, DDM/rhBMP-2 release, rhBMP-2.

INTRODUCTION

Bone grafts are the biomaterials used for bone augmentation. Bone graft materials must present one of the regenerative properties- osteogenicity, osteoconductivity, osteoinductivity. Autograft, Allograft, Xenograft and Alloplast are four types of bone graft materials currently available. Many of these graft material lacks osteoinductive potential despite being osteoconductive and biocompatible. Among all, an autograft is considered a gold standard, because it perceives all three regenerative properties.[1] Advantage of autogenous graft is rapid healing time, no risk of immune reaction or graft rejection. However, it has some disadvantages like it requires an additional donor surgery site, risk of related site morbidity, harvests a limited amount of bone and longer surgery time.

Demineralized dentine matrix (DDM) is a good alternative as a graft substitute as it is biocompatible along with osteoinductive and osteoconductive potential. Several studies showed similarity in chemical composition between DDM and demineralise bone matrix (DBM).[2] Urist was one of the first scientists who observed bone inducing property of rabbit dentine in 1967.[3] First clinical report on human dentine autograft was performed in 2002 in Japan for maxillary sinus lifting case and was reported in 2003,(81th IADR Sweden).[4] DDM is becoming highly popular in dentistry as a graft material for various purposes like socket preservation, guided bone regeneration, maxillary sinus lift etc. Bone morphogenetic proteins (BMPs) are water-soluble proteins due to which they rapidly dissolved and cannot be utilised alone to its full potential. BMPs are first identified in dentine and from the demineralized bone.[5] BMP-2 have a strong bone inducing property among the BMPs isolated and has been accredited by the food and drug administration (FDA) and is commercially available since 2002.[5]

The rhBMP-2 induces pluripotent mesenchymal stem cell to differentiate into osteoblast and chondrocyte separately.[6] In 2007, United States (US) FDA accredited the rhBMP-2 (in the concentration of 1.5mg/ml) along with absorbable collagen sponge (INFUSE BONE graft, Medtronics, Memphis, TN, USA) as an alternative to autogenous bone graft for the localised augmentation of alveolar bone.[7]

The approved concentration of rhBMP-2, 1.5mg/ml is around 10^6 times higher than naturally secreted in the human body.[8] Inflammatory side effects due to the increase of osteoclastic activity can be seen in the cancellous bone environment due to the high concentration of rhBMP-2[1,9]. A good scaffold is needed which ensures slow and sustained release of rhBMP-2 rate. DDM with its micro porous structure can be a good scaffold for rhBMP-2 for hard tissue regeneration. Properties of both combine and act more efficaciously.

Requirements of scaffold for rhBMP-2 carrier

For the carrier of rhBMP-2, a scaffold should meet the following requirements.[9-13]

1. Maintaining a certain rhBMP-2 concentration in the grafted area for a sufficient time to enhance the activity of the protein for new bone formation
2. Good harmony between BMP and its scaffold to maintain sustained and prolonged release of rhBMP-2.
3. Biocompatible, ease of sterilisation and biodegradability with low immunogenicity.
4. Adequate porous structure for cell infiltration and vascularisation.
5. Keep the biological activity of rhBMp-2.
6. Have adequate compressive and tensile strength.
7. Easily available.

However, an ideal carrier system has not been identified until now for the application of rhBMP-2 as a part of the regenerative procedure.

Scaffolds for rhBMP-2 carrier

An extensive range of investigations both experimentally and clinically have been done for the development of an organic and inorganic type of scaffolds which can act as a carrier of rhBMP-2.[14-17] various types of the scaffold that have been investigated are-

Organic scaffolds- organic scaffolds consist of two groups biological and synthetic. The biological group have; demineralized bone matrix, demineralized dentine matrix, collagen and fibrin. The synthetic group, which includes polylactic acid (PLA), poly lactic-co-glycolide (PLGA) and hydrogels.

Inorganic scaffolds- Comprises of; hydroxyapatite, tricalcium phosphate, bioceramics and metals. There are also some other inorganic scaffolds which have undergone limited evaluation; viz: Coral, natural bone mineral, non-demineralised bone particles, polyphosphate polymer.

Composite scaffolds- a combination of the two; organic and inorganic scaffolds termed composite scaffold are; Collagen-HA, Gelain/TCP, TCP-Collagen, TCP-HA, HA-Coral.

The advantage of inorganic scaffolds is they are structurally strong, immunologically inert, osteoconductive and variably biodegradable. At the present time collagen and synthetic polymer scaffolds offer the greatest potential for clinical use.

Comparison between different scaffolds materials is given in Table-1.

Table-1 Comparison of Dose and release of BMP-2 using four major material as a carrier system.

Categories	Sub-Categories	Scaffold	BMP-2 release	Key results
Inorganic Scaffolds	_	Hydroxyappatite granules	10microgram	Non-significant
		Beta-TCP granular implant	1-10mg	Better fusion rate
Organic Scaffolds	Biological	Collagen	1microgram	Improved bone fusion rate
		Chitosan	5microgram	Capable to adapt bone area
	Synthetic	PLA	100-800mg/g	rhBMP-2 depends on the dose
		PLGA	3.5microgram,17.5microgram	No synchronization
Composite materials	_	Collagen-HA	10 microgram	Anabolic and catabolic agent
		Gelatin/beta-TCP	5 microgram	Bone formation higher in the low p-TCP content

In 1998 it was suggested that human root dentine prepared from the extracted tooth can be used as a carrier of rhBMP-2 as it induces new bone formation in the periodontium.[18] A later report in 2005 showed that DDM particles have an osteoinductive property and it can act as a carrier of rhBMP-2 for bone engineering.[10-21] DDM has shown that it has a great potential to deliver the rhBMP-2 successfully. Various studies have shown that DDM has a micro porous structure with dentinal tubules which contribute not only to the loading of rhBMP-2 but also with a sustained and efficient release. Along with the osteoconductive and osteoinductive properties of DDM Adequate porosity of DDM helps in cell and blood vessel penetration. DDM also have adequate mechanical stability to withstand compression and tension, biodegradability, adhesiveness to adjacent bone, and the most importantly to retain the protein for a sufficient time.[5,17,20-21]

Preparation of Demineralized Dentin Matrix for the delivery of Recombinant Human Bone Morphogenetic Protein-2 (DDM/rhBMP-2)

Preparation of demineralized dentin matrix

Autotooth derived graft material was first prepared in Korea tooth bank R & D centre in Seoul in 2009.[22] It is divided into two types: block-type and powder type according to its manufacturing process.[22] There are several approaches through which Demineralised dentine matrix can be prepared. Extract the natural tooth, clean it and then crushing it through several methods like- a) conventional hand-operated stainless steel[23], b) Using tooth mill24(osteomill, Tokyo iken, co ltd) at 12000rpm for 30 sec, c) Percussion mill25 (Polymix Px-MFC90 D, d)Kinemetica AG, Switzerland), e) Smart dentine grinder (kometa bio). The size of the particles can range from 300 to 1500 microns. Thereafter, dentine powder can be demineralized. The demineralization time ranged from 30 minutes to 24 hours.[26] 0.6N HCl[24] ,0.34N HNO3 for 30 minutes[27], 2% HNO3 for 10 minutes. The most commonly used demineralizing agent is 2%HNO3 for 10 minutes. Ethylene diamine tetra acetic acid (EDTA) can also be used for demineralization.[7,19,27,28] EDTA treated dentine is named as Treated dentine matrix(TDM).[26,28] Partially demineralised dentine matrix is 70% demineralised dentine. The term demineralized dentine matrix and partially demineralised dentine matrix are used interchangeably in almost all reports because DDM is always partially demineralized.[25-26]

Conformation of demineralised dentine matrix

Demineralised dentine matrix and demineralised bone matrix have a similar chemical composition consisting of 18% Collagen mainly type I, 2% non-collagenous proteins like Phosphophoryn and sialoproteins (trigger bone remodelling) and 70% of hydroxyapatite and body fluid (10%) in weight volume.[29]The matrix also acts as a repository of many growth factors. Some of the growth factors are; bone morphogenetic proteins (BMPs), Transforming growth factor-beta, insulin-like growth factor (IGF), basic fibroblast growth factors.[24,30] Beesho et al, in 1991 have isolated the BMP from the human dentine matrix for the first time.[31] this dentine matrix derived BMPs are similar to Bone derived BMPs and have presented with an identical function in vivo.[29] BMP-2, BMP-4 and BMP-7 are present in dentine matrix and termed endogenous BMPs (ED-BMPs). DDM contains dentinal tubules (1-3 micrometre in diameter) in number of 18000-21000 (tubules/mm2)[32] with the volume porosity on an average of 3.47%±1.46% which is lower, in comparison to volume porosity of natural human bone (6.2%).[33] dentinal tubules plays a crucial role in the release of endogenous growth factors present in the matrix. Various studies on dentine collagen have shown that it exhibits superior cell adhesion, tissue compatibility, absorptivity and low antigenicity.[29] X-ray diffraction (XRD) analysis has also revealed that the low crystalline structures, domain sizes, and high Ca/P dissolution of the dentine matrix are similar to those of autogenous bone. These included HA, TCP, amorphous calcium phosphate, and octacalcium phosphate with the plate-like crystals[20].

The embodiment of rhBMP-2 with DDM (DDM/rhBMP-2)

The embodiment of DDM and rhBMP-2 is achieved by two methods. The first is by Physical adsorption, suggested by Luginbuel et al, considered as the simplest method for delivering proteins.[34] In this approach, DDM scaffold is immersed into the protein solution, which is then allowed to dry and achieves the physical adsorption by placing rhBMP-2 into the interfibrillar space and the dentinal tubules of the DDM. Porosity influences physical adsorption. With demineralisation, dentinal tubules of DDM is enlarged to form pores of around 3-3.5 microns similar to natural human bone pore size.[33,35]

Secondly, the Modified physical entrapment, that is accomplished by combining a phase changing liquid scaffolding material along with rhBMP-2. This phase change of gelation causes the physical entrapment of proteins within the carrier.[36] Freezing increases shrinking and decrease the melting of collagen fibres. This results in the entrapment of rhBMP-2 deeply into the dentinal tubules Nanoporous region and in the interfibrillar spaces.

Several studies regarding DDM as rhBMP-2 carrier are summarized in Table-2

Table-2 The preparation of DDM and DDM embodiment with rhBMP-2.

DDM source	Subjects	Carrier type	Dimineralization	rhBMP-2 dose and embodiment method	Reference
Human tooth root	mice , athymic mice/hindquarter muscle	Dentine block	0.6NHCL (PDM)	1, 2, and 5 mg rhBMP-2/ 70 mg DDM: NA	(18)
Rat tooth root	rats /palatal connective tissue	Dentine block	24% EDTA (NA)	50 and 100 mg/mL rhBMP-2: soaking	(37)
Human tooth	rats /subcutaneous pocke	Dentine powder	0.6 N HCl (CDM)	5.0 mg BMP-2/70 mg DDM: soaking	(5)
Rabbit tooth	Rabbits /calvarium	Dentine powder	0.6 N HCl (PDM)	50 mg rhBMP-2/300 mg DDM: freeze-drying	(38)
Rabbit tooth	Mice /thigh muscle	Dentine powder	0.6 N HCl (PDM)	50 mg rhBMP-2/300 mg DDM: freeze-drying	(39)
Rabbit tooth	Rabbits /calvarium	Dentine powder	0.6 N HCl (PDM)	50 mg rhBMP-2/300 mg DDM: freeze-drying	(39)
Human tooth	Humans/alveolar bone	Dentine powder	0.6 N HCl (PDM)	0.2 mg/mL rhBMP-2: freeze-drying	(40)
Human tooth	Humans/alveolar bone	Dentine powder	0.6 N HCl (PDM)	0.2 mg/mL rhBMP-2: freeze-drying	(41)
Human tooth	Humans/alveolar bone	Dentine powder	0.6 N HCl (PDM)	0.2 mg/mL rhBMP-2: freeze-drying	(35)

Studies on DDM/rhBMP-2

(Abbreviations CDM (completely demineralized dentin matrix); DDM (demineralized dentin matrix); EDTA (ethylenediaminete traacetic acid); PDM (partially demineralized dentin matrix); ABB(anorganic bovine bone),NA (not available); rhBMP-2 (recombinant human bone morphogenetic protein)).

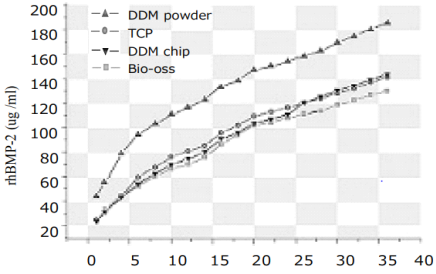
Young-Kyun kim et al compared the rhBMP-2 release kinetics from four different graft materials that is 1) Bio-OSS, 2) tricalcium phosphate, 3) DDM powder, 4) DDM chips, in vitro for over 36 days (graph-1).

DDM Powder formed by pulverisation (0.5-1.0mm) of extracted human teeth And DDM chips is formed by cleaning of extracted tooth and then sectioning at a cementoenamel junction and after that making holes of diameter 0.2mm from the teeth surface to the pulp chamber at

equidistant of 0.5 to 1.0mm of the teeth surface. then it was washed with ethyl alcohol and decalcification done in 0.6N HCL for 2 hours. bone chips of sizes of size 1.0-3.0mm×1.0mm were formed from the decalcified teeth block and then again demineralized, defatted and dehydrated to reduce the mineral content to less than 5% by weight.

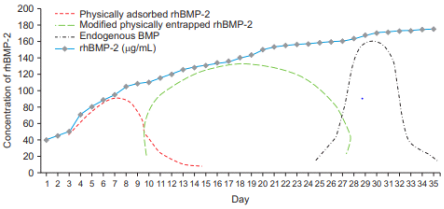
Among the four graft substitute the burst release was greatest for rhBMP-2 in DDM powder and the amount of rhBMP-2 released by remaining three grafts were similar to one another. Over the course of the 36-day experimental trial, all four grafts showed a sustained and gradual release of rhBMP-2 (graph-1). As compared to other graft materials, DDM powder has shown a statistically significant larger amount of rhBMP-2 release. Of all the materials tested, DDM may thus be the most appropriate rhBMP-2 vehicle for hard tissue regeneration.[20]

Comparison of release kinetics of rhBMP-2 from four different graft materials over the time period of 36 days given in graph-1

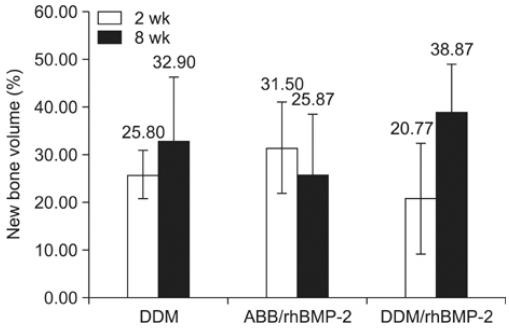


Graph-1. Comparison of release kinetics of rhBMP-2 from four different graft materials over the time period of 36 days.[20]

Pietrzak et al proposed the release of endogenous BMP-7 in two-compartment. Rapidly released during the first hour, followed by a sustained and slower release up to 168 hours. Early release of BMP-7 from loose compartment and storage of BMP-7 in the tight compartment is responsible for sustained and slow-release, resulting in long term retention inside the DDM carrier.[42] According to their proposal, Um et al also suggested a tripartite pattern of rhBMP-2 release from DDM carrier.43 In the First stage, early release of physically adsorbed rhBMP-2 occurred from the loosened interfibrillar space pores (graph-2, red line) and promoted by phenotypic changes in fibroblast to produce osteoblast. Deeply entrapped rhBMP-2 is released by collagen degradation (graph-2, green line) through dentinal tubules and interfibrillar space during the second stage. Eventually, in the later stages, The release of endogenous BMPs (graph-2, black line) from DDM is initiated due to resorption of the mineralized core of DDM during the remodelling process which is modulated by various GFs within the dentine mineral phase (graph-2). Postulated release kinetics of rhBMP-2 from DDM given in graph-2.



Graph-2 Postulated release kinetics of rhBMP-2 from DDM.[43]



Graph-3 Comparison of new bone volume formation with DDM, DDM/rhBMP-2, and ABB/rhBMP-2.[38]

In the rat calvarial defect, DDM group showed a slight increase whereas rhBMP-2 combined DDM group showed a marked increase in bone volume over a period of two to eight week. In contrast, anorganic bovine bone (ABB) combined with rhBMP-2 showed decrease bone volume at 8 weeks compared to earlier stages. Suggesting DDM was a better rhBMP-2 carrier than ABB/rhBMP-2.[38] (graph-3)

Comparison of new bone volume formation with DDM, DDM/ rh BMP-2, and ABB/rhBMP-2 shown in graph-3

In 2005 Murata et al, performed a study in nude mice and showed that DDM can act as a bone inducing material and suggested DDM can act as a unique, absorbable and osteoconductive matrix carrier of BMP-2 for bone engineering.[19,44]

Kim et al, in their study involving 23 patients with 36 implants have confirmed the healing potential of DDM/rhBMP-2. The finding revealed the desirable osseointegration in 35 implant sites concerning good implant stability and low marginal bone loss.[45-46]

In another clinical study performed by, Woong Um et al showed the effect of DDM in socket preservation techniques after tooth extraction. The amount of new bone formation was 34.39% with DDM/rhBMP-2 and 29.75% with DDM; the amounts of residual dentin were 8.35% and 16.15% respectively. Suggesting DDM as a potential carrier for rhBMP-2.[35]

Above studies discussed have showed that DDM a good carrier of rhBMP-2. rhBMP-2 can not be used alone as it is water soluble and dissolved rapidly so a carrier is required to transfer the rhBMP-2. Greater concentration due to early release of rhBMP-2 also induces the inflammatory reactions so the scaffold should have porous structure which allow the sustained and slow release of the protein. DDM have most of the properties to act like a suitable carrier as it has adequate mechanical strength to withhold mechanical and tensional forces generated, absorbable providing the osteoconductive property, osteoinductive, microporous structure that helps in loading of rhBMP-2 and also in sustained graded release to use at its maximum potential. Properties of both DDM and rhBMP-2 acts synergistically and maximize the outcome. Clinical application of DDM/rhBMP-2 have been done in guided bone regeneration (GBR), Socket preservation, localised ridge augmentaion and maxillary sinus lifting procedures and gave the promising results.

CONCLUSION

This review, suggest that DDM is a good carrier for rhBMP-2 showing favourable results with bone formation and both acts reciprocally. This review also describes the release profile of rhBMP-2 from DDM/rhBMP-2 that occurs in a controlled and sequential tripartite pattern. This reduces the local complications and is effective at a low concentration of rhBMP-2, thus DDM can be regarded as a potential rhBMP-2 carrier.

Further studies should be focused on the suitable concentration, incorporation technique, and precise release kinetics of rhBMP-2 and DDM.

Furthermore, using DDM as a stem cell carrier for alveolar bone regeneration will be a huge step forward in dentistry.

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