A Case Report of Allogenic Demineralized Dentin Matrix loaded with Recombinant Human Bone Morphogenetic Proteins for Alveolar Bone Repair

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Introduction

The purpose of this case report is to introduce the effectiveness of allogenic demineralized dentin matrix (Allogenic DDM) loaded with recombinant human bone morphogenetic proteins (rhBMP-2) for alveolar bone repair. Bone-inducing agent, rhBMP-2, was locally needed to stimulate the native bone healing capacity because the alveolar socket to be repaired seemed to be critical-sized defect. The clinical findings with respect to the healing process were that there were no remarkable inflammation and immune rejection that impair the healing process and are coincident with those of the previous studies. The nanopore structure of dentinal tubules in unique avascular and acellular Type I collagenous dentin matrix seems to make it feasible to carry and release rhBMP-2 effectively on local site based on the previous study. For the purpose of making reliable conclusions, this preliminary case report must be led by the retrospective case series of the allogenic DDM loaded with rhBMP-2(Allogenic DDM/rhBMP-2).

Abstract

The aim of this case report was to introduce the effectiveness of allogenic demineralized dentin matrix (Allogenic DDM) loaded with recombinant human bone morphogenetic proteins (rhBMP-2) for alveolar bone repair. Bone-inducing agent, rhBMP-2, was locally needed to stimulate the native bone healing capacity because the alveolar socket to be repaired seemed to be critical-sized defect. The clinical findings with respect to the healing process were that there were no remarkable inflammation and immune rejection that impair the healing process and are coincident with those of the previous studies. The nanopore structure of dentinal tubules in unique avascular and acellular Type I collagenous dentin matrix seems to make it feasible to carry and release rhBMP-2 effectively on local site based on the previous study. For the purpose of making reliable conclusions, this preliminary case report must be led by the retrospective case series of the allogenic DDM loaded with rhBMP-2(Allogenic DDM/rhBMP-2).

Keywords: Allogeneic DDM, Demineralized dentin matrix (DDM), Recombinant human bone morphogenetic proteins (rhBMP-2)
However, dentin and bone have similar components that consist of 10% body fluid, 20% organic materials, and 70% minerals as mainly hydroxyapatite (HAp), and contain bone morphogenetic proteins (BMPs) that is very important for the reprocessing procedures of demineralization or decellularization in terms of safety and effectiveness [13,14]. While DDM can be considered similar reprocessed tissue scaffolds, it results in the elimination of the major part of the mineral phase and the immunogenic components of dentin matrix, but retains collagen that provides a structured osteoconductive scaffold and a soluble protein fraction comprising several growth factors (GFs), BMPs among them [15]. Consequently, many researchers have suggested by in vivo and in vitro experiments that DDM might be effective materials as osteoconductive and osteoinductive collagenous carriers of exogenous rhBMP-2 for alveolar bone repair [16-19].

The purpose of this preliminary case report is to introduce the healing capacity of allogenic DDM/rhBMP-2 in critical-sized alveolar bone defect by clinical observation and histological evaluation. The authors hypothesized based on the previous study that the allogenic DDM with nanopore dentinal tubules sized 1.0-3.0 μm diameter might be effective carrier for rhBMP-2 with little or no immune responses of antigenicity that would otherwise impair the clinical healing process.

Clinical Case

Fabrication of allogeneic DDM powder (processed by Korea Tooth Bank)

For the DDM powder, tooth was selected that was stored in Korea Tooth Bank with donation consent and screening. Tooth root dentin was pulverized (0.3-0.8 mm) after removing the attached soft tissue and pulp. The root dentin powder was washed using ethyl alcohol and demineralized for 30 minutes in 0.6 N HCl by repeated 30-minute periods and then dehydrated, defatted, and freeze-dried to reduce the mineral content to less than 10-30% by weight, and leaving type I collagen as the main constituent [20]. (Korea Patent Number 10-1062381)

Fixation of rhBMP-2 to allogeneic DDM powder (the dip-dry method)

The rhBMP-2 was loaded to the DDM powder by placing 2 mg/ml rhBMP-2 (Cowell, Busan, Korea) and 0.03 g of DDM powder into individual 15ml conical tubes. The mixtures were frozen in a deep freeze at -70 ºC, slotted into a lyophilization glass bottle, and then fixed in a lyophilizer (ILShin Lab, Seoul, Korea) [17].

Case Report

A 45-year-old woman presented with a selfexfoliated implant with 3 year old history on her upper left first molar on October 2014. Two months later, she encountered a same problem on her upper right first molar implant (Figure 1). The clinical finding on upper right first molar was large, crater like critical-sized defect which might be impossible to install implant with appropriate stability (Figure 2a). The defect

Figure 1: Panoramic view. Two months before spontaneous exfoliation of upper right first molar implant (red arrow) that encountered exactly same as upper left first molar.

Figure 2: Clinical features and Cone Beam Computed Tomography (CBCT).

a: Large, crater like critical-sized defect remained two months after implant exfoliation.
b: The defect was repaired by Moldable Allogenic Demineralized Dentin Matrix loaded with Recombinant Human Bone Morphogenetic Proteins (allogenic DDM/rhBMP-2).
c: About 12 months after, the surgery site was well organized and bony structure were filled in the defect.
d: The type of defect illustrated in CBCT showed aseptic loosening rather than infectious process.
e: Allogenic DDM/rhBMP-2 can be seen on the alveolar crest area in CBCT.
f: About 12 months after graft, allogenic DDM/rhBMP-2 was transformed into dense cortical and cancellous bone complex in CBCT.
was scheduled to be repaired by moldable type Allogenic DDM/rhBMP-2 as described elsewhere for easy handling and insertion into the remained buccal cortical rim and alveolar socket (Figure 2b) [21]. After the graft, all the healing process was uneventful and the allogenic DDM/rhBMP-2 was well incorporated within the defect through continuous remodeling. About 12 months later, when the flap was reflected to install the implant, that was well organized, bony structure was found on the alveolar crest to be able to place the implant easily (Figure 2c). Tissue from healed site was procured by trephine drill for the histological evaluations before implant installation.

The type of defect in cone beam computed tomography (CBCT) before graft revealed aseptic loosening rather than infectious process due to extraordinary masticatory forces on the implant (Figure 2d) [22]. Allogenic DDM/rhBMP-2 grafted on the alveolar crest area maintained its form and shape very well in CBCT (Figure 2e). About 12 months later; the CBCT revealed the completely healed and organized alveolar bone with dense cortical and cancellous bone that could be a parameter for healing and remodeling capacity (Figure 2f) [6].

The histological evaluation at the time of the implant placement 12 months after allogenic DDM/rhBMP-2 graft showed that there was newly formed bone around DDM power with abundant blood vessels which might be organized into bone marrow. Remained particle was embedded and closely contacted with newly formed bone which has osteocytic lacunar with blood vessels that might be evidences of remodeling (Figure 3).

**Discussion**

The purpose of this preliminary case report is to introduce the effectiveness of the healing capacity of allogenic DDM/rhBMP-2. The authors hypothesized that allogenic DDM has the capacity for carrying rhBMP-2 with little or no immune responses of antigenicity that would otherwise impair the clinical healing process, based on the previous in vitro and in vivo studies as well as clinical outcome of familial tooth bone graft which means allogenic DDM graft between biologic family members [17-19,23-25].

As for the BMP carrier, it was known that collagen is the most documented carrier for rhBMP-2, but it is not osteoconductive and is not well suited for onlay augmentation owing to its poor structural integrity in alveolar bone. However, DDM has excellent structural integrity and mechanical properties with its own hydroxyapatites (HAPs), type I collagens, and additional dentin matrix proteins.

Ike, et al. and Murata paid attention to the possibilities of DDM as BMP carrier. Ike et al. in 1998 reported that allogenic partially demineralized dentin matrix (allogenic PDM/rhBMP-2) induces heterotopic bone formation with dose dependent curves and PDM provided the mineral and matrix for adsorption of rhBMP-2. So that exogenous rhBMP-2 adsorbed onto a pulverized root PDM proved to be as osteoinductive as autogenous bone [16]. Murata in 2005 also showed that human DDM particles are osteoinductive, insoluble collagenous matrices and the DDM might be effective as a carrier of rhBMP-2 for bone engineering [13].

Kim, et al. compared the releasing kinetics and osteonectin expression from three different scaffolds loaded with rhBMP-2 such as tricalcium phosphate, inorganic bovine bone and human DDM. The most large and slow release of rhBMP-2 was expressed in the DDM scaffold and the higher expression of osteonectin from DDM exhibited significant mature bone formation. They concluded that human DDM powders appear to have great potential as an effective scaffold for rhBMP-2 [17]. Kim, et al also reported that human DDM/rhBMP-2 showed more bone formation than TCP/rhBMP-2 in dorsum muscle pouch of nude mice and Um et al reported similar results with Kim et al in rat calvarial defect consecutively [18,19]. The first human clinical application of autogenous DDM/rhBMP-2 with successful result was reported by Kim in 2014 [26].

With regard to the allogenic DDM, many researchers have already performed in vitro and in vivo experimental studies for examining the osteopromotive properties and immunologic reactions of allogenic DDM. In the 1970s, Bang, et al. performed several animal studies and observed that the inflammatory reaction was negligible in the allogenic dentin either demineralized or denaturalized/lipophilized [9,10]. These results were in accordance with the Urist’s observation that a second set of implants of demineralized, lyophilized allogenic bone had induced bone formation almost as well as the first set [27]. Recently, Gomes, et al. explained that the absence of rejection of the allogenic DDM corroborated the previously reported results of Carvalho et al. that appeared to be a consequence of the low antigenicity of allogenic DDM [28,29].

Regarding clinical applications of human allogenic DDM, earlier in 1975, Nordenram indicated that the use of allogenic DDM in the treatment of 33 human jaw cysts had a lower and higher incidence of operational defect and complete healing, respectively, upon radiologic examination confirmation [11]. In 1983, Schwartz clarified that no clear correlation could be demonstrated between the incidence of immune reactions and the clinical/radiographic course of allogenic DDM treatment, neither could any correlation be demonstrated between the immune reactions and the HLA- or ABO- matched grades. However, a detectable immunological reaction is attributable to the cytoplasmic membrane antigens; in part the odontoblastic processes in dentin and in part the cementocyte membranes.
in cementum [30]. In 1986, Fugazzotto et al. also strongly suggested that freeze-dried dentin merited consideration as an osteoinductive agent in 16 cases of human periodontal osseous defects [12]. In 2014, Um, et al. reported on the treatment outcomes of familial tooth, block type allogenic DDM, following a successful sinus augmentation with simultaneous implant placement. Consequently, the successful long-term result of this application was reported in 2015 [24,25].

The histological specimen and the radiologic evidences of this case report indicated that the bone forming capacity of allogenic DDM/rhBMP-2 was not inferior to that of the autogenous DDM and autogenous DDM/rhBMP-2 and that the occasional immunizing effects of allogenic DDM may have constitute no theoretical contraindication for its a further clinical applications [6,26]. While it was presumed that an alloreactive glycoprotein in DDM was derived from presence of odontoblastic process or cementocyte membranes, extraction of possible histocompatibility antigens without influencing on the osteoconductive capacity of DDM was not unattainable due to acellular, avascular nature of DDM [30].

This preliminary clinical report introduces that the healing capacity of allogenic DDM/rhBMP-2 may be as effective as that observed with autogenous DDM and allogenic DDM is potential carrier of rhBMP-2. However, further retrospective case series must be needed to confirm the safety and effectiveness of allogenic DDM/rhBMP-2.

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Conflict of Interest

The authors have no conflicts of interest to declare.

Abbreviations

CBCT: Cone Beam Computed Tomography

DDM: Demineralized Dentin Matrix

rhBMP-2: Recombinant Human Bone Morphogenetic Proteins

References


