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Root coverage achieved with a connective tissue graft overlaid onto allogeneic demineralized dentin matrix incorporated with rhBMP-2

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Running title : Root coverage with allo-DDM, rhBMP-2 and connective tissue

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ABSTRACT

Root coverage achieved with a connective tissue graft overlaid onto allogeneic demineralized dentin matrix incorporated with rhBMP-2

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Autogenous demineralized dentin matrix (DDM) has been widely used in implant dentistry and highlighted as a potential carrier of recombinant human bone morphogenetic protein-2 (rhBMP-2). Allogeneic DDM (Allo-DDM) is comparable to autogenous DDM in safety and effectiveness. The various endogenous growth factors in dentin, including BMP, transforming growth factor, fibroblast growth factor, and insulin growth factor suggest that DDM incorporated with rhBMP-2 (DDM/rhBMP-2) may exert a synergistic effect that promotes soft tissue healing through angiogenic activity, in addition to its osteoinductivity. This case report describes a successful outcome of root coverage where connective tissue graft (CTG) was overlaid onto Allo-DDM incorporated with rhBMP-2 (Allo-DDM/rhBMP-2).

Key words : demineralized dentin matrix; connective tissue graft; fistula closure; dentistry; root coverage

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I . Introduction

Bone morphogenetic proteins (BMPs), which are capable of osteoinduction, were first identified in 1965 by Marshall Urist in demineralized bone matrix and dentin[1]. Several members of the BMP family have been separated and human recombinant BMP-2 (rhBMP-2) has been produced for therapeutic applications. In 2007, the US Food and Drug Administration (FDA) granted approval of rhBMP-2 (1.5 mg/mL concentration) to be used with a collagen sponge[2]. However, the burst release of the rhBMP-2 can cause tissue edema, erythema, and ectopic bone formation due to lack of mechanical stability of the collagen sponge when exposed to blood[2].

Extensive research in tissue engineering has been undertaken to overcome the complications of this rhBMP-2 carrier, including substituting biomaterials, polymers, composites, and hydrogels[3]. Demineralized dentin matrix (DDM) consists of a highly cross-linked type I collagen with non-collagenous matrix-binding proteins (NCPs) such as BMPs, transforming growth factors (TGFs), fibroblast growth factor (FGF), and insulin growth factor (IGF)[4]. Among many NCPs, endogenous dentin BMPs, which are similar to bone matrix-derived BMPs, have been identified and purified, showing similar actions in vivo[5]. Dentin matrix contains various minerals, such as physiological calcium phosphate (hydroxyapatite and β-tricalcium phosphate; HA and β-TCP), which exhibits diverse resorbable property, resulting in enhancement of the degradation of dentin collagen[6]. DDM, with its nano-porous structure (dentinal tubules and inter-fibrillar space) and endogenous dentin-derived BMP, has been highlighted as a potential carrier of rhBMP-2[7]. DDM incorporated with rhBMP-2 (DDM/rhBMP-2) showed enlarged dentinal tubules and osteoclastic resorption of the dentin surface as well as osteoinductive bone formation at the early stage, and the resorption lacuna was filled with new bone regeneration[7]. In addition to BMPs that induce angiogenic response, and several growth factors encourage soft tissue healing[4,8].

Modern dentistry is more focused on patient's expectations. As dental implant treatment has become the gold standard for rehabilitation of missing tooth, many patients and clinicians readily decide to extract teeth, where alveolar bone and gingiva have been destructed, to prevent further bone loss. However, root coverage can be operated to fulfill aesthetic and functional requirement in order to maintain teeth. Connective tissue graft with a bilaminar technique such as advanced gingival flap has been suggested as standard protocol for root coverage especially in the anterior maxilla, as known as aesthetic zone, in terms of long-term stability and predictability. Despite most of regenerative surgery for root coverage has been focused on the soft tissue healing, it is evident that the main cause lies in alveolar bone loss[9]. The necessity of novel method has been constantly raised regeneration bone as well as gingiva with consider to the innovative development the bone graft materials or scaffolds incorporated with a growth factor. This paper highlights the clinical outcome of Allo-DDM/rhBMP-2 root coverage with CTG.

Ⅱ. Case presentation

A 39-year-old male visited our hospital, complaining of a mucogingival fistula on right maxillary canine area(Fig. 1). He was a non-smoker, had no systemic diseases and took no medication. Radiographic examination revealed a cystic lesion on the right maxilla and the size was about 1cm x 1cm. Since the patient denied to extract the other teeth which could be fabricated for Auto-DDM, he was scheduled for the cyst enucleation and root coverage with CTG and Allo-DDM/rhBMP-2.

To preserve right maxillary canine (#6), the treatment plan was to close the fistula and to repair the alveolar bone and gingiva around right maxillary canine(Fig. 2). Allo-DDM/rhBMP-2 was prepared (0.8cc Allo-DDM; HuBT, Korea Tooth Bank, Seoul, Republic of Korea) with freeze-dried rhBMP-2 (0.2mg/ml, Cowellmedi, Seoul, Republic of Korea) in individual 15-ml conical tubes. The mixtures were left to freeze at -70°C, slotted into a lyophilization glass bottle and then dried in a lyophilizer (Ilshin Lab, Seoul,Korea)[10]. After triangular incision and full-thickness flap elevation, the cystic lesion was enucleated with apicoectomy of the right maxillary canine. The exposed root was treated with a tetracycline-soaked cotton pellet to improve results of the root coverage(Fig. 2A)[11]. Approximately 0.7cm x 1cm CTG was harvested from the ipsilateral hard palate(Fig. 2B). The hydrated Allo-DDM/rhBMP-2 was grafted into the cystic defect and on the exposed root surface until the root was fully covered (Fig. 2C, 2D). CTG was then carefully placed on the covered graft(Fig. 2E), and fixed at the recipient site using 5-0 nylon(Fig. 2F).

At 1 week after surgery, the stitches were removed without any complications(Fig. 3A). The grafted CTG was healed and survived 1 month post-surgery (Fig. 3B). Five months after the surgery, fully organized mucogingival tissue was maintained with new

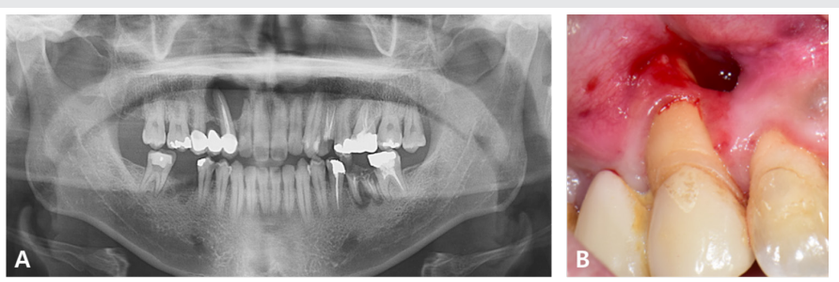

Figure 1. Pre-operative clinical and radiographic images. A. Cystic lesion on the right maxillary canine. B. Severe gingival recession on the right maxillary canine and fistula on the center of cystic lesion.

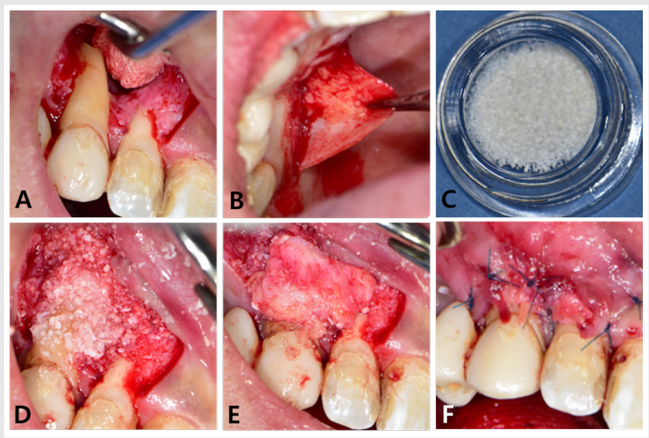

Figure 2. Surgical procedure for root coverage using allogeneic demineralized dentin matrix incorporated with recombinent human bone morphogeneic protein-2 (Allo-DDM /rhBMP-2) and connective tissue graft (CTG). A. After cyst enucleation and apicoectomy, the root surface of maxillary canine was treated with tetracycline. B. Connective tissue was harvested from ipsilateral hard palate. C. Allo-DDM/rhBMP-2. D. Allo-DDM/rhBMP-2 was packed into cystic defect and covered the root. E and F. The harvested CTG was carefully covered on the Allo-DDM/rhBMP-2. and fixed with 5-0 nylon. Allo-DDM/rhBMP-2; Allogeneic DDM incorporated with rhBMP-2, CTG; Connective tissue graft.

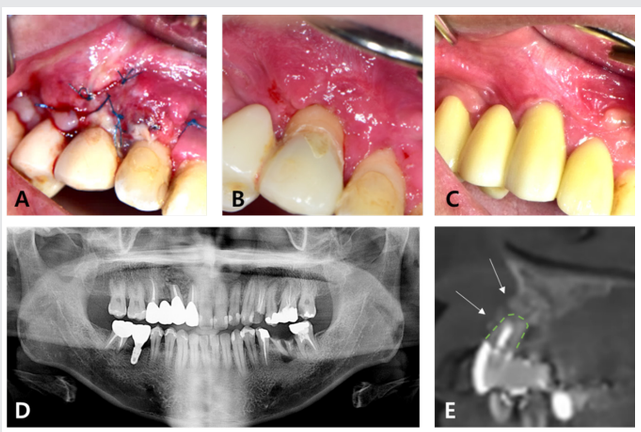

Figure 3. Post-operative images. A. Uneventful healing of gingiva at 1 week after the surgery. B. Gingiva recession was occurred, the soft tissue was healed without dehiscence at 1 month postoperative. C. At postoperative 5 months, there was uneventful healing with new prosthesis. D. At postoperative 5 months, postoperative panoramic x-ray showed normal healing state on the surgical site without recurrence of the cystic lesion. E. At postoperative 5 months, postoperative cone beam computed tomography showed that the DDM (arrow) had covered the labial root surface of maxillary canine (#6) (spotted line).

prosthesis(Fig. 3C). Radiographic exam showed that cystic defect was repaired successfully and labial root of right maxillary canine was well covered with the graft material(Fig. 3D and 3E).

Ⅲ. Discussion

To the best of our knowledge, this is the first report of simultaneous application of Allo-DDM/rhBMP-2 and CTG for root coverage to repair both alveolar bone and gingiva. Although CTG was overlaid onto the DDM particles, the grafted soft tissue healed without necrosis or graft failure. In addition, the right maxillary canine (#6) was maintained without any symptoms, and the DDM grafts were surrounded on the root in CBCT at five months postoperatively. Regarding the clinical and radiological outcome at the last follow-up, it appears that complete remodeling of grafted DDM was not achieved yet. However, the patient, who wanted to prevent extraction of his own tooth, was satisfied with the results both aesthetically and functionally.

In 2017, DDM with rhBMP-2 (0.2mg/mL of rhBMP-2, Cowellmedi, Busan, Korea) was first used for socket preservation and demonstrated more embedding osteocytes with active bone formation in comparison to DDM alone[10]. In 2019, the release profile of rhBMP-2 incorporated with DDM suggested slow and sequential releasing of total BMP, including the endogenous BMP[12]. In 2020, DDM was suggested as a suitable carrier of rhBMP-2 based on histological review even though a low dose of rhBMP-2 (0.2mg/mL) was coated on the DDM[10,13]. The endogenous growth factors in DDM include TGF-β1 as the most abundant growth factor; FGF2, BMP-2, IGF-1 and vascular endothelial growth factor (VEGF) as the intermediate amounts; and BMP-4 and BMP-7 as the least abundant[4]. Among these, FGFs, PDGF, and VEGF promote differentiation and growth of various cells for soft tissue healing, such as fibroblasts and osteoblasts[14]. TGF-β1 has the broadest spectrum of actions in several processes of wound healing such as angiogenesis, inflammation, fibroblast proliferation, and collagen synthesis, affecting all cell types, involved in all stages of soft tissue healing. TGF-β1 can rapidly recruit leukocytes to accumulate and initiate the inflammation phase. During this phase, some inflammatory cytokines are secreted to reach the inflammatory response within a few days. As the inflammation subsides, the tissue formation is occurred with re-epithelialization and granulation tissue formation. A series of biological reaction acts to promote the granulation formation, including fibroblast activation, extracellular matrix production, and neovascularization[15]. Neovascularization, the growth of new blood vessels, is an important component of soft tissue healing[16]. An important property of FGF and VEGF is the temporarily increase of angiogenesis in the area of soft tissue healing. Two mechanisms of neovascularization are vasculogenesis and angiogenesis. Vasculogenesis is the de novo formation of new vessels by endothelial progenitor cells. Angiogenesis is observed frequently during soft tissue repair. New vessel growth is led by a single endothelial cell known as the tip cell, which direct

vascular outgrowth by sensing proangiogenic mediators like VEGF[17]. VEGF has been discussed as a potential therapy for intractable field to enhance angiogenesis, and also vasculogenesis, by the more formation of blood vessels and improvement of the blood circulation in the area of the wound.

With regarding soft tissue healing, in 2022, DDM graft showed successful clinical results with uneventful soft tissue healing and 76.7% of bone healed without primary closure[18]. Um et al.(2020)[7] reported that rhBMP-2 incorporated into DDM may initiate both osteoinductive bone formation and osteoclastic resorption of the dentin matrix simultaneously. Furthermore, dentin has many types of other NCPs, which are known to influence cell behavior, including dentin sialoprotein, osteopontin, dentin matrix protein-1, and matrix extracellular phosphoglycoprotein[4,19,20]. As a result, quantification of osteoclastic resorption behaviors revealed an 11-fold higher resorption on dentin compared with bone[21]. Through this osteoclastic resorption, NCPs could be easily released than DDM alone or could not be released until degradation or resorption of dentin matrix. This osteoclast-related release of NCPs could be a type of dual delivery system. Release of NCPs as matrix- and mineral-binding proteins could then initiate healing process to facilitate bone remodeling as well as soft tissue regeneration[7]. Thus, both rhBMP-2 and NCPs in DDM exerted a positive effect on neovascularization and tissue maturation in this patient.

To the best of our knowledge, the treatment of gingival recession has traditionally focused solely on addressing the soft tissue, as covering the underlying bone carries a significant risk of infection or bone loss[9]. However, the authors of this study suggested the application of Allo-DDM on the exposed root, which has shown promising results in achieving substantial root coverage. There were some limitations due to retrospective designed case report such as small numbers of patient, short follow-up period, absence of histological analysis and periodontal examination, and insufficient information before the surgery. Within the limitations, the authors note, however, that this case report can only go so far in identifying the effect DDM/rhBMP-2 on soft tissue healing, as there was no control group, histologic and periodontal examination, short-term follow up, and small numbers of patient.

Successful root coverage was achieved with free connective tissue graft (CTG) overlaid onto the Allo-DDM. Since DDM has plenty of endogenous growth factors including bone inducing and soft tissue healing, the DDM induced engraftment of the connective tissue through the growth factors that promote angiogenesis and osteoinductivity. Regarding the healing process, rhBMP-2 could be affect easily releasing the endogenous growth factors in DDM. Further study should be conducted to evaluate the soft tissue healing capacity of DDM/rhBMP-2.

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