Clinical Report

Autograft of Demineralized Dentin Matrix Prepared Immediately after Extraction for Horizontal Bone Augmentation of the Anterior Atrophic Maxilla: A First Case of Non-Vital Tooth-Derived Dentin

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Abstract: Onlay bone augmentation is a challenging field, especially in esthetic zones. In implant dentistry, labial bone loss is generally recovered through autologous bone and/or biomaterials. Dental pioneers have been applying a patient-own demineralized dentin matrix (DDM) for bone regeneration. The aim of this study was to histologically and radiologically evaluate horizontal bone augmentation on an anterior atrophic maxilla using an autograft of non-vital tooth-derived DDM for implant. A 56-year-old female patient presented with severe labial bone loss. Autologous DDM was immediately prepared for horizontal ridge augmentation. DDM blocks were fixed with titanium screws for lateral augmentation. DDM granules were grafted on the atrophic bone between the DDM blocks. Twelve months postoperatively, bone biopsy was performed from the implant placement point for histological observation. X-ray computed tomography (CT) was performed before and after the DDM graft surgery. Hematoxylin and eosin sections showed that new bones were directly generated on the DDM residue. CT images showed the original buccal bone lines and root-type and wall-type DDM. The grafted DDMs were clearly found on the original bone. The outlines in the 22nd region indicated an 8.11 mm horizontal width after the implant placements compared to 4.95 mm before the augmentation. Additionally, the width at the upper level increased from 3.38 mm to 5.92 mm. DDM scaffolds contributed to the horizontal bony support required to place the implants. The patient experienced therapeutic success with DDM immediate autograft and implant surgery.

Key words: Dentin, DDM, Autograft, Bone augmentation, Atrophic maxilla

Introduction

Extracted teeth are usually discarded as potentially infectious medical waste. However, dental pioneers have been applying a patient-own demineralized dentin matrix (DDM) for bone regeneration1-3. Following reports of the bone growth-inducing properties of rabbit DDM in 19674, 5, the first clinical trial of a human DDM autograft for bone augmentation of the sinus floor was successfully conducted in 20026, 7. The DDM granules were derived from non-functional vital upper molars for implant placement. To avoid donor site morbidity to harvest autologous bone, non-functional teeth were recycled as auto-biomaterials for bone regeneration6.

Dentin and bone have similar chemical composition1. 7. Their components (in weight volume) include hydroxyapatite (HAp; 70%), collagen (18%), non-collagenous proteins (NCPs; 2%), such as bone morphogenetic proteins (BMPs), and body fluid (10%). Rabbit demineralized bone matrix (DBM) granules induce the growth of bone and cartilage in muscle5. Fresh and properly prepared DBM and DDM perform better in osteoinduction than highly calcified tissues such as normal dentin and cortical bone1. 9. It is considered that hydroxyapatite crystal structures inhibit the release of various growth factors representative of BMPs in the mineralized matrix9. There is growth factor activity in vital tooth-derived DDM even after demineralization. However, non-vital tooth-derived DDM loses BMP activity through the action of protein-fixation drugs such as Form Cresol (FC) during root-canal treatments. Therefore, non-vital tooth-derived DDM can be defined as a cross-linked collagenous matrix without growth-factor activity9. Collagen is a major component of connective tissue in the human body. Dentin collagen is significantly more insoluble than bone and skin collagen. We focused on the characteristics of naturally cross-linked, slowly soluble dentin collagen for the width-maintenance of horizontal bone augmentation. In this report, non-vital and hopeless teeth were recycled as DDM scaffolds (granule-type, root-type, and wall-type), and DDM immediate autograft (Fig. 1) was prepared for horizontal bone augmentation in the esthetic zone for implant placement. Bone augmentation with autologous DDM was estimated histologically and radiologically. To our knowledge, this is the first case report to utilize non-vital tooth-de-
Materials and Methods

Patient

The patient was a 56-year-old female who presented with missing teeth (11, 21) after the upper anterior bridge was broken in 2015 (Fig. 2a). A clinical examination revealed highly atrophic bone in the maxillary anterior zone. Her past medical history was insignificant.

First surgical operation

X-ray computer tomography (CT) (Veraviewepocs, J. Morita corp., Osaka, Japan) was performed before the DDM graft surgery. Under the intravenous sedation, tooth (12, 22, 23) were extracted, based on the oral examination and the treatment planning (Fig. 2b). Metal cores and debris were removed carefully from the non-vital teeth (12, 22) as possible. The three extracted tooth were divided into a granule-type DDM and a wall-type DDM (Fig. 2b). The extracted lateral incisor (12) was crushed with saline ice blocks using exclusive teeth mill (Osteo-Mill, J. Morita corp., Osaka, Japan) at 12,000 rpm for 30 sec. Next, the crushed tooth-granules were demineralized in 2% HNO$_3$ for 20 min. The extracted lateral incisor (22) and canine (23) were fixed in the medical device (FIX-kun, J. Morita corp., Osaka, Japan) at 12,000 rpm for 30 sec. Next, the crushed tooth-granules were demineralized in 2% HNO$_3$ for 30 min. All DDM materials were rinsed in cold saline for 10 min, and put in the sterilized dish with autologous platelet-rich fibrin (PRF) prepared from the intravenous blood (Fig. 2c).

The extracted sockets were filled with HAp granules (Apaceram AX, size 0.6-1.0 mm, porosity 85%, HOYA technosurgical Co., Ltd, Tokyo, Japan). Cortical perforations were added into the atrophic bone, and immediately grafted-DDM blocks were fixed by titanium screws (Fig. 2d and 3a, b). The HAp and DDM granules were covered with PRF and collagen membrane (Jason membrane, Botiss biomaterials GmbH, Zossen, Germany) (Fig. 3c), and sutured with polytetrafluoroethylene threads (GORE-TEX Suture, W.L. Gore & Associate, Inc., Delaware, USA) (Fig. 3d). The patient took antibiotics for 4 days and there was not a complication.

Second surgical operation

In 2016, mucoperiosteal flap was elevated under the intravenous sedation, and bone biopsy was done from the implant placement point for histological observation. Then 4 fixtures (12 :Legacy-2 SBM, 11.5 mm length, 3.7 mm diameter, 21 :Legacy-4 HA, 11.5 mm length, 3.7 mm diameter, 22 :Legacy-3 SBM, 11.5 mm length, 3.7 mm diameter, 23 :Legacy-2 HA, 10 mm length, 3.2 mm diameter, Implant Direct Corporation, California, USA) were implanted into the augmented bone (Fig. 4a), and sutured with PTFE threads. X-ray CT was performed just after the implant surgery. Six months later, final crowns were set (Fig. 4c).

Tissue preparation for HE sections

The tissue was fixed in 10% formalin and decalcified in 10% formic acid. After that they embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE) staining.

Ethical considerations

The clinical study concept and the informed consent forms for the surgical procedures performed with this study were approved by the ethical committee for human subjects at Health Sciences University of Hokkaido (No. 29) in conformity with the principles of the Declaration of Helsinki.

Figure 1. Appearances of DDM. a: Macroscopic observation of wettable granule-type DDM, b: SEM of granule, c: Macroscopic observation of wettable root-type, d: SEM of root-type.
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Figure 2. Case: 56 year-old woman, extraction and augmentation by DDM (surgical procedure 1). a: Initial view before surgery, Note: atrophic bone, b: View just after extraction, c: Autogenous DDM and platelet rich fibrin (PRF), d: Immediate graft of wall-type DDM.

Figure 3. Augmentation by DDM (First surgical operation). a, b: View of lateral augmentation, HA granules in sockets, c: Sockets covered with PRF, d: Sutured with PTFE streads.
Figure 4. View after placement of fixtures (surgical procedure 2) and biopsy tissue. a: Placement of fixtures into augmented bone, b: new bone connected directly with DDM (HE staining), c: Final panoramic X-ray photography.

Figure 5. X-ray CT before and after augmentation by DDM in 56 year-old woman. a, c: X-ray CT and oral view before surgery, b, d: X-ray CT and oral view after augmentation and fixture placement.
Figure 6. Comparison of Initial X-ray CT images (left) and X-ray CT images at 12 months (right) after DDM graft. a, b: White distance indicating width before and after surgical procedure (12), a: fractured tooth, b: Arrows showing original labial bone line. c, d: White distance indicating width before and after surgical procedure (11), d: Arrow head showing DDM. e, f: White distance indicating width before and after surgical procedure (21), f: DDM supporting fixture, Arrow head showing DDM. g, h: Non-placement area (21-22). White distances indicating width before and after surgical procedure, g: Note: atrophied bone, h: Horizontal augmentation by DDM (arrow head). i, j: White distances indicating width before and after surgery (22), i: Note: atrophied bone, j: DDM (arrow head) supporting fixture.
of Helsinki.

**Results**

**Histological findings**

New bone was formed directly on the DDM residue. The boundary line between the DDM residue and bone was clearly seen, and acellular spaces of dentinal tubes were observed in the DDM (Fig. 4b).

**X-ray CT assisted analysis**

Fig. 5 shows the horizontal X-ray CT images and the intraoral views before tooth extraction (Fig. 5a, c) and after implant placement (Fig. 5b, d). Fig. 6 shows the sagittal outlines of the anterior maxilla before and after bone augmentation with the DDM. The white lines indicate the bone width before and after surgery. Fig. 6a, b show the outlines in 12 regions, indicating a 6.09 mm horizontal width after surgical procedure 2 compared to 4.07 mm before surgical procedure 1. Fig. 6c, d show the outlines in 11 regions, indicating a 5.66 mm horizontal width after surgical procedure 2 compared to 2.93 mm before surgical procedure 1. The X-ray CT image in Fig. 6d shows the grafted wall-type DDM on the original bone. Fig. 6e, f show the outlines in 21 regions, indicating a 6.26 mm horizontal width after surgery 2, compared to 2.70 mm before surgery 1. The implanted fixture was covered with bone-like tissue. Fig. 6g, h show the outlines in the 21-22 region, indicating a 6.42 mm horizontal width after surgery 2 compared to 2.48 mm before surgery 1. Grafted root-type DDM was clearly found on the original bone. Fig. 6i, j show the outlines in 22 regions, indicating an 8.11 mm horizontal width after surgery 2 compared to 4.95 mm before surgical procedure 1. Additionally, grafted root-type DDM fixed with a titanium pin was observed apart from the fixture in Fig. 6j, and the width at the upper level increased from 3.38 mm to 5.92 mm.

**Discussion**

Biomaterials have strong potential for improving patient quality of life when applied to treatment. Various natural and synthetic biomaterials have been developed by designing and constructing scaffolds for bone regeneration. In recent years, the field of tissue engineering has focused on absorbable biomaterials; non-absorbable materials have not been harmonized with bone remodeling. We have been striving to develop DDM scaffold designs using ultrasonic acid-etching technology for structural modification. Ultrasonic demineralization resulted in increase in cracks and surface area of dentin scaffolds, providing cellular-differentiation spaces. Ultrasonically demineralized bone plates induced bone growth subcutaneously at 2 weeks, while a calcified bone plate failed to induce bone growth until 6 weeks. These reports and others suggested that the processing procedure of demineralization provides graft-materials, which are derived from hard tissue, a prominent bone regenerative potential compared to that of non-treated highly calcified graft-materials.

The X-ray CT images after the second surgical operation showed more remarkable horizontal augmentation than those before the first surgical operation (Fig. 6). This was a case of a distinctive DDM onlay graft combined with three types of DDM (root, wall, and granule) for lateral ridge augmentation. DDM scaffolds were received by the host himself, and four fixtures were able to place into the augmented ridge. Therefore, we believe that DDM scaffolds contributed to the sufficient temporary mechanical support required to place the implants. DDM and DBM are materials derived from natural sources. Both DDM and DBM are degraded biologically by enzymes, leading to their eventual metabolism and bone remodeling, which confers on them a distinct advantage over synthetic materials. DDM and DBM are composed mostly of acid-insoluble type I collagen (95%), and the remainder (5%) is made up of NCPs. In our previous report, human DDM and human DBM had a capacity of inducing bone and cartilage independently at 4 weeks in the subcutaneous transplantation of nude mice, similar to BMP-induced cellular differentiation at ectopic sites. In the present case, we confirmed that newly-formed bone directly combined with DDM in the biopsy specimen. The bonding line between DDM and bone was clear, and the dentinal tube spaces of DDM were free of bacteria. Moreover, cartilage and multinucleated giant cells were not seen in the specimen. The patient experienced a therapeutic success with DDM immediate autograft and implant surgery.

Recently, several clinical reports have used autogenous non-demineralized tooth materials in bone defects, autogenous partially demineralized dentin for sinus augmentation, and allogenic DDM. Impacted intact wisdom teeth were crushed into granules using bone mills and hammers. The whole tooth-derived granules were grafted in two bone defects after the removal of radicular cysts. The non-demineralization method has the advantage of being associated with a shorter surgery duration. However, the strong acid treatment for dentin has several advantages and 2% HNO3 solution (pH < 1.0) can kill bacteria and decellularize organs during the demineralization process of the extracted tooth. Therefore, the preparation method is aseptic and associated with decreased antigenicity. The DDM has better osteo-induction performance than calcified dentin. Besides the osteo-inductive property, the DDM can also activate the coagulation cascades of blood plasma components. The clot enhancing reaction of blood plasma by DDM is advantageous for surgical operations and retains various paracrine growth factors, which should be beneficial for post surgical healing.

Structural factors influence the capability of biomaterials for tissue regeneration. Human root-type DDM scaffolds with artificial large size pores in sheep iliac bone defects results in bone ingrowth into the pores and bony bridges between the pores. A compact poreless structure inhibits cell migration and blood supply into the inside of the biomaterials and on the contralateral side. These structural contexts induce a negative effect termed “biomaterial wall” denoting the exclusion of cells and blood supplies. Dentin possesses an ultra-fine structure with dentinal tubes. The natural dentin structures comprise a markedly suitable scaffold for tissue regeneration; additionally, ultrasonic processing and the provision of artificial large-size pores could modify dentin into a three-dimensional scaffold of greater functionality, which coordinate the bio-absorption rate and the adsorption ability for proteins and cells.

Recently, DDM transplantation has been becoming a standard technique in South Korea. We have considered non-functional teeth as innovative non-utilization natural resources and have proposed for medical recycling of patient-own teeth as novel suitable biomaterials for tissue regeneration. We expect that this dentinal collagen-based regenerative therapy will become a dental innovation in the 21st century. Biomaterial science in dentistry should further support and develop advanced regenerative therapeutic solutions using dentin-derived biomaterials for periodontal regeneration.

DDM is defined as an acid-insoluble dentin collagen with natural cross-links and comprises a cell-free absorbable extracellular matrix with a dentinal tube-derived ultra-fine structure. The DDM scaffold contributed to sufficient horizontal support, which is required for implant placement. The patient experienced therapeutic success with DDM immediate autograft and implant surgery. To our knowledge, this was the first case to utilize a non-vital hopeless tooth-derived DDM for the purpose of horizontal ridge augmentation. Wisdom tooth- or hopeless
tooth-derived DDMs could be reused as block- or granule-type materials based on physician judgment even if the teeth from which the DDM autografts derive are non-vital or infected.

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Conflicts of Interest

The authors have declared that no COI exists.

References

1. Murata M, Akazawa T, Mitsugi M, Kabir M, Minamida Y, Um I, Kim K, Kim Y, Sun Y and Qin C. Autograft of dentin materials for bone regeneration. In: Advances in Biomaterials Science and Biomedical Applications, ed by Pignatello R, INTECK Publisher, 2013, pp 391-403.

2. Akazawa T, Murata M, Hino J, Nakamura K, Masahiko K, Masaharu M and Um IW. Surfaces design and functional control of demineralized dentin matrix granules derived from human teeth. In: Advances in Oral Tissue Engineering, ed by Murata M and Um I, Quintessence Publishing Co, Inc, 2014, pp 37-42.

3. Kim Y, Lee J, Kim K, Um I and Murata M. Healing mechanism and clinical application of autogenous tooth bone graft material. In: Advances in Biomaterials Science and Biomedical Applications, ed by Pignatello R, INTECK Publisher, 2013, pp 404-435.

4. Yeomans JD and Urish MR. Bone induction by decalcified dentine implanted into oral, osseous and muscle tissues. Arch Oral Biol 12: 999-1008, 1967.

5. Bang G and Urish MR. Bone induction in excava-tion chambers in matrix of decalcified dentin. Arch Surg 94: 781-789, 1967.

6. Murata M. Autogenous demineralized dentin matrix for maxillary sinus augmentation in human. The first clinical report. 81th International Association for Dental Research, Geteburg, Sweden, 2003.

7. Murata M, Akazawa T, Mitsugi M, Um I, Kim K and Kim Y. Human dentin as novel biomaterial for bone regeneration, In: Biomaterials-Physics and Chemistry, ed by Pignatello R, INTECK Publisher, 2011, pp 127-140.

8. Urish MR. Bone: formation by autoinduction. Science 150: 893-899, 1965.

9. Huggins C, Wiseman S and Reddi AH. Transformation of fibroblasts by allogeneic and xenogeneic transplants of demineralized tooth and bone. J Exp Med 132: 1250-1258, 1970.

10. Murata M, Okubo N, Shakya M, Kabir MA., Yokozeki K, Hino J, Naka-mura K, Ishikawa N, Shibata T and Arisue M. Bone induction of human tooth and bone crushed by newly developed automatic mill. J Ceram Soc Japan 118: 434-437, 2010.

11. Murata M, Inoue M, Arisue M, Kubo Y and Nagai N. Carrier-dependency of cellular differentiation induced by bone morphogenetic protein in ectopic sites. Int J Oral Maxillofac Surg 27: 391-396, 1998.

12. Murata M, Sato D, Hino J, Akazawa T, Tazaki J, Ito K and Arisue M. Acid-insoluble human dentin as carrier material for recombinant human BMP-2. J Biomed Mater Res A 100: 571-577, 2012.

13. Arabadzhiev I, Maurer P and Stevao E. Particulated wisdom teeth as an autologous bone substitute for grafting/filling material in bone defects: Case Report. J Clin Exp Dent 12: e424-e428, 2020.

14. Minamizato T, Koga T, I T, Nakatani Y, Umebayashi M, Sumita Y, Ikeda T and Asahina I. Clinical application of autogenous partially demineralized dentin matrix prepared immediately after extraction for alveolar bone regeneration in implant dentistry: a pilot study. Int J Oral Maxillofac Surg 47: 125-132, 2018.

15. Kim Y, Bang K, Masaru. Murata, Masaharu. Mitsugi and Um I. Retrospective clinical study of allogenic demineralized dentin matrix for alveolar bone repair. J Hard Tissue Biol 26: 95-102, 2017.

16. Fujita M, Uchara O, Miyakawa H, Naito S, Kabir M, Murata M and Nakazawa F. Detection of bacteria in human tooth-derived biomaterials. In: Advances in Oral Tissue Engineering, ed by Murata M and Um I, Quintessence Publishing Co, Inc, 2014, pp 31-32.

17. Huggins CB and Reddi AH. Coagulation of blood plasma of guinea pig by the bone matrix. Proc Natl Acad Sci USA 70: 929-933, 1973.

18. Reddi AH. Bone matrix in the solid state: geometric influence on differentiation of fibroblasts. Adv Biol Med Phys 15: 1-18, 1974.

19. Kuboki Y, Saito T, Murata M, Takita H, Mizuno M, Inoue M, Nagai N and Poole AR. Two distinctive BMP-carriers induce zonal chondrogenesis and membranous ossification, respectively; geometrical factors of matrices for cell-differentiation. Connect Tissue Res 32: 219-226, 1995.

20. Kabir M, Murata M, Akazawa T, Kusano K, Yamada K and Ito M. Evaluation of perforated demineralized dentin scaffold on bone regeneration in critical-size sheep iliac defects. Clin Oral Implants Res 28: e227-e235, 2017.

21. Kabir M, Murata M, Shakya M, Yamada K and Akazawa T. Bio-absorption of human dentin-derived biomaterial in sheep critical-size iliac defects. Materials (Basel) 14(1): 223, 2021.

22. Kim YK, Kim SG, Byeon JH, Lee HJ, Um IU, Lim SC and Kim SY. Development of a novel bone grafting material using autogenous teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109: 496-503, 2010.

23. Kim Y, Um IW and Murata M. Tooth bank system for bone regeneration - Safety report -. J Hard Tissue Biol 23: 371-376, 2014.
