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Dentin Materials as Biological Scaffolds for Tissue Engineering

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Abstract

Vital tooth-derived demineralized dentin matrix (DDM) has a bone-inductive ability, while non-vital tooth-derived DDM lost it. Acid treatment for dentin provides the increase of surface area, the release of matrix-binding growth factors such as BMPs, and the decrease of the infection risk. Human autograft of vital tooth-derived DDM was achieved first in Japan 2002, while first bone autograft was noted in Italy 1820. This paper introduced dentin/bone biology and a unique clinical case, combined with two types of non-vital tooth-derived DDM (roots, granules) for lateral bone augmentation. A 63-year-old woman revealed highly atrophic mandible in 2015. Three non-vital teeth were extracted, changed in shape, demineralized in 2% HNO₃, were rinsed, and were grafted immediately. The CT images at 3 months after the graft showed remarkable lateral augmentation. DDM scaffolds were received to host, and two fixtures were placed into the DDM-augmented bone. The patient was successfully restored with their own DDM scaffolds and implant surgery.

Keywords: dentin, bone, material, scaffold

1. Introduction

Regenerative medicine is based on applied biomaterial science. Biomaterials have a strong impact on the patient cure for improving the quality of life. Recently, absorbable materials have been needed in the field of tissue engineering. We have been challenging to develop bioabsorbable dentin scaffolds [1–13], harmonized with bone remodeling, by using the ultrasonic acid-etching technology [14–17] (**Figure 1**).

2. History of bone graft

First bone graft was allograft from dog to human in 1682. While xeno- or allograft of bone was a major in the Western countries during the Middle Ages, human bone autograft was noted first in 1820, Italy. The use of bovine-demineralized bone matrix (DBM) was reported first in 1889. The xenogenic DBM was grafted into human skeletal defects caused by osteomyelitis. Many DBM papers were published in the twentieth century [18]. Generally, fresh autogenous bone remains the gold standard of treatment for nonunion and large bone defects [19].

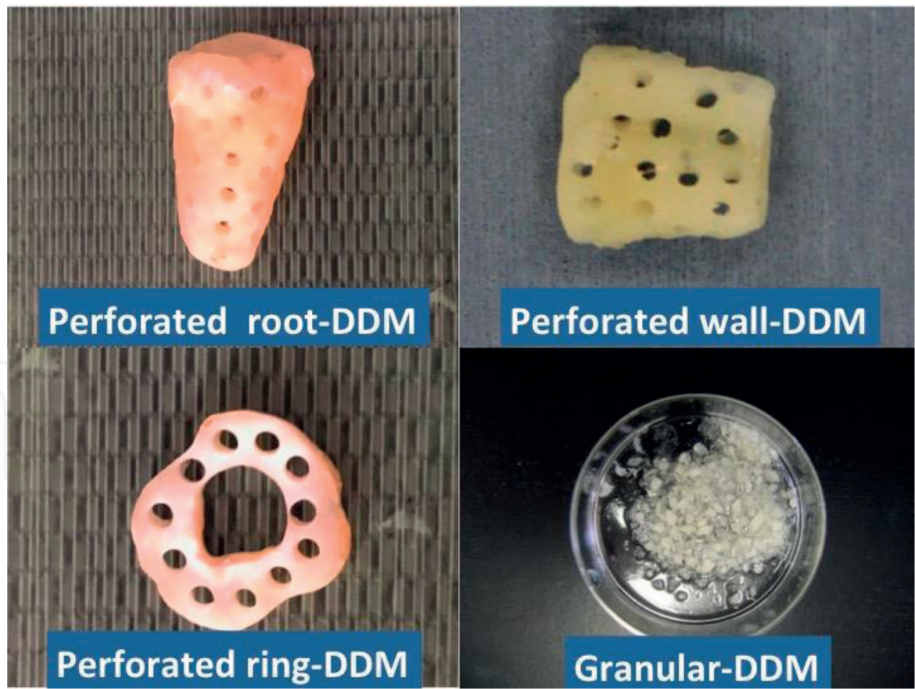


Figure 1.
Types of dentin materials.

However, cortical bone block inhibits the invasion of cells and blood vessels, because the calcified structure is highly dense like a castle wall [20].

3. History of dentin graft

First dentin autograft in human was achieved in 2002 in Japan for bone augmentation and reported in 2003, 81th IADR, Sweden [21]. The first clinical case was a sinus lifting, using demineralized dentin matrix (DDM) granules, derived from nonfunctional vital upper molars (#17, embedded #18) for implant placement [21]. We consider that the nonfunctional teeth are medical resources and can be recycled for transplant and bone regeneration. Recently, DDM graft has become a standard technique in South Korea. The unique service system in Korea Tooth Bank is the preparation and delivery of the tooth-derived materials on demand [4, 7, 8]. The tooth-derived materials were named as auto-tooth bone (ATB), which is divided into the block-type and powder-type [22]. The demineralized dentin, hydrated in 0.9% NaCl solution for 15–30 min before use, can be cut by operators with a surgical knife or scissors. We have thought of nonfunctional teeth as innovative resources and have advocated the medical recycle of the patient’s own tooth as novel materials for bone regeneration. This matrix-based therapy is “*dental innovation*,” early in twenty-first century. Our advanced technique will expand from East Asia to the world, and dentin graft has been becoming a realistic alternative to the bone autograft.

4. Characteristics of dentin and bone

Dentin and bone are mineralized tissues. Dentin is cell-free matrix without a blood vessel, while bone includes osteocytes and vessels. However, dentin and bone are almost the same in chemical components. They consist of biological apatite (HAp: 70%), collagen (18%), non-collagenous proteins (NCPs: 2%), and body fluid (10%) in weight volume (**Table 1**). BMPs and FGFs are matrix-binding proteins,

	Inorganic	Organic	Fluid	BMP
Dentin	70	20	10	+
Cortical bone	70	20	10	+
Cartilage	2	28	70	+
Ceramics	100	0	0	-
Collagen	0	100	0	-

Table 1.
Chemical components (wt/v%) of human dentine and bone.

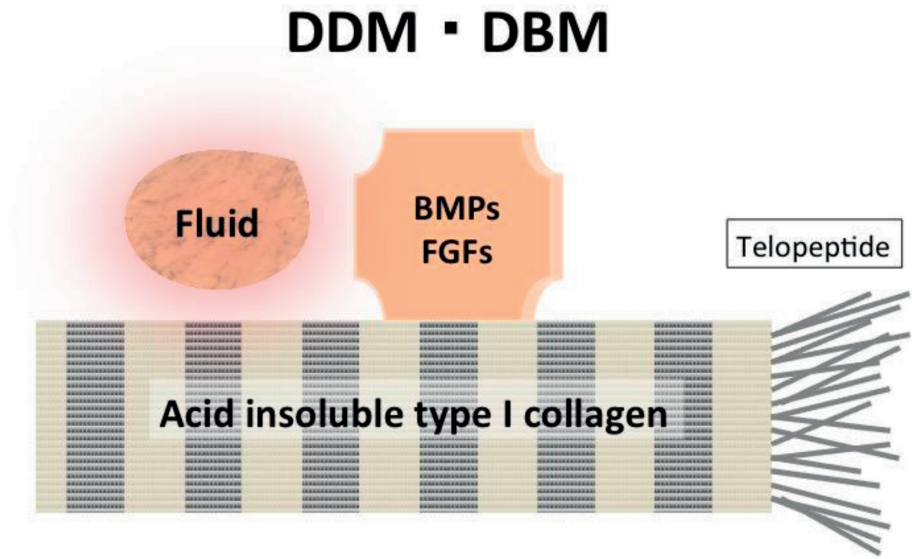


Figure 2.
DDM and DBM. BMPs and FGFs, matrix-binding proteins in NCPs. Collagen, mainly type I collagen.

while OCN is a mineral-binding protein in NCPs [13]. The small integrin-binding ligand, N-linked glycoprotein (SIBLING) family, including dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), and osteopontin (OPN), are secreted into the ECM during the biomineralization [23–26]. The SIBLING phosphoproteins (DSPP, DMP1, BSP, OPN) bind to titanium beads [27]. DDM and DBM are mainly type I collagen (95%); the remaining is made up of NCPs, including a small amount of growth factors. Briefly, DDM and DBM are acid-insoluble collagen-binding bone morphogenetic proteins (BMPs) [28–30] and fibroblast growth factors (FGFs) [31–34] (**Figure 2**).

5. Advantages of demineralization

There are several advantages about demineralization process of dentin and bone. In our preparation, 2% HNO₃-demineralized dentin (DDM) is sterile material. The strong acid solution (pH < 1) can kill bacteria and decellularize organ. Therefore, the strong acid treatment has antiseptic properties and decreases antigenicity. Bacteria-free DDM was detected after the culture for 7 days in blood

agar medium of dentin granules demineralized in 2% HNO_3 for 20 min [35]. HAp crystals inhibit the release of BMPs along with growth factors [36]. After the removal of HAp crystals by ultrasonic demineralization, the surface area of dentin and bone increases remarkably [16]. In our study, human DDM and human DBM induced bone and cartilage independently at 4 weeks in the subcutaneous tissues of nude mice [34]. In addition, adult rat cortical bone plate treated with ultrasonic demineralization induced bone at 2 weeks, while fresh cortical bone plate never induces bone until 6 weeks [20]. These results indicated that highly calcified tissues such as cortical bone and calcified dentin did not have a better capability in bone induction than DDM and DBM. The delayed inductive properties of highly calcified dentin and bone may be related to the inhibition of BMP release by HAp crystals [36]. We never think, therefore, fresh bone is a gold standard. DDM and DBM have a better performance in bone induction than fresh dense bone.

6. Ultrasonic demineralization for dentin scaffold

Dense structure without pores inhibits the cell invasion and the body fluid permeation into the inside of the biomaterials. This situation is so-called material's wall. Material's wall means the exclusion of cells and body fluid. Dentin has compact structure with dentinal tubes. We have been challenging to create biological dentin scaffolds, using ultrasonic demineralization technology [14, 16, 37]. The whole structure design of dentin by the artificial pores and the ultrasonic treatment might produce functional 3D scaffolds, which control the bioabsorption rate and the adsorption ability for proteins and cells [2, 38] (**Figure 1**). The innovative technology can create the adequate 3D geometry and the surface structure of commercially available materials [14, 15] (**Figure 3**). Geometrical factors will improve the performance of biomaterials for bone regeneration [39–42].

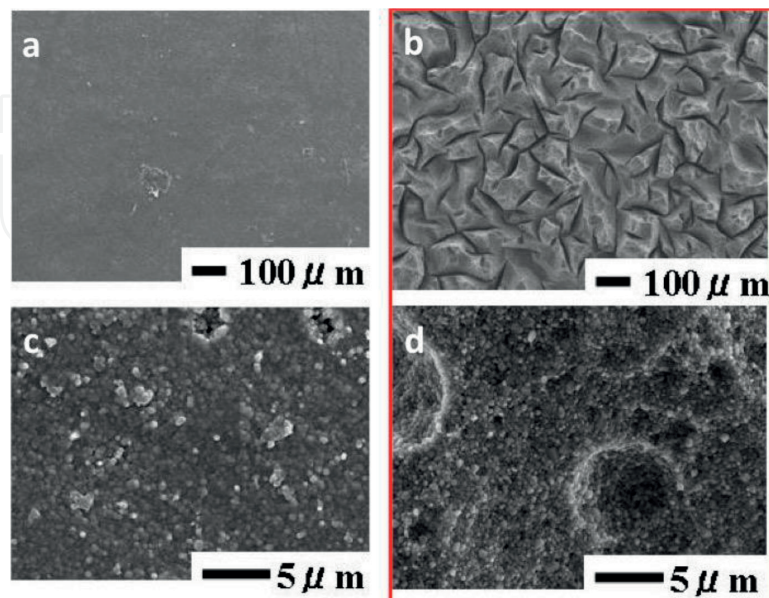


Figure 3. SEM views of HAp dense plate before and after dissolution for 20 min in 2.0% HNO_3 by ultrasonic treatment at 600 W and 28 kHz. (a, c) Normal HAp dense plate (Cell-yard®); (b, d) ultrasonic demineralized plate; (a) dense flat plate; (b) knife cut-like grooves; (c) dense round crystals; (d) crater-like holes.

7. Biochemistry of DDM and DBM

Both DDM and DBM are composed of predominantly type I collagen (95%) and matrix-binding proteins such as BMPs [39, 43, 44]. BMPs bind to type I collagen of dentin and bone, even after complete demineralization (**Figures 1 and 2**). The fact is a reason why DDM and DBM induce bone and cartilage. Completely demineralized rabbit dentin matrix induced bone in the muscle at 4 weeks, while calcified dentin induced bone at 8–12 weeks after implantation [45, 46]. Many researchers made effort to discover dentin-derived BMPs [31, 47–49]. In 1990, BMPs, transforming growth factor beta (TGF- β), insulin growth factor-I (IGF-I), and IGF-II, were detected in human dentin [50]. Moreover, DDM and DBM possess the ability to coagulate blood plasmas [51]. The coagulation action of blood plasma by DDM should become advantageous for surgical operations. Interestingly, antibacterial activity within degradation products of biological scaffolds composed of extracellular matrix was published [52]. Additionally, extracellular matrix extracts from the dentin and pulp showed antibacterial activity against three types of anaerobic bacteria associated with dental disease [53].

8. Dentin scaffolds

DDM is defined as an acid-insoluble dentin collagen with natural cross-links and is a cell-free absorbable biomatrix with dentinal tube structure. DDM from autogenous tooth can be applied for bone grafts and tissue engineering as its own biomaterial, thus allowing improvement of bone induction while reducing the risk of infection. Human DDM can be recycled as cross-linked collagen in familial graft and allograft, because DDM is a cell-free matrix without antigenicity. We published bone regeneration in sheep iliac bone defect by human DDM root-type scaffold or β -TCP block [2, 54] (**Figure 4**). Bovine- and pig-derived collagenous materials are available as medical devices for the human body all over the world.

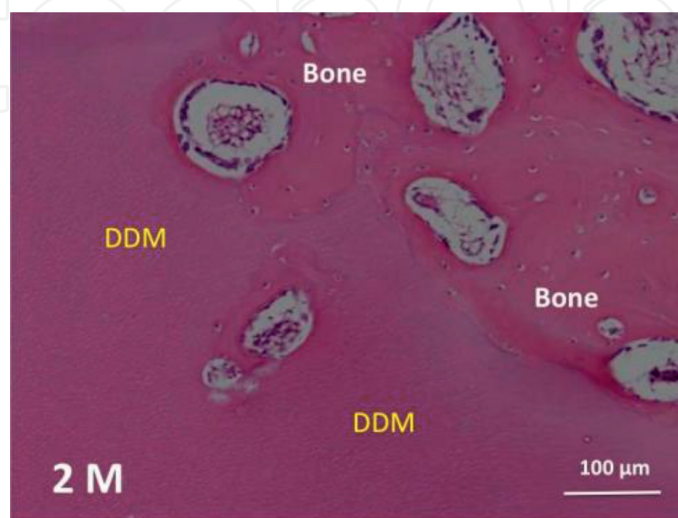


Figure 4.
Human DDM scaffold in sheep iliac bone defect at 2 months (HE).

9. Comparison of dentin scaffolds and biomaterials

Dentin scaffolds are composite materials of organic and inorganic components with natural cross-linking. Active 3D structure can be created in dentin scaffolds by using ultrasonic demineralization method and/or handmade technique under doctor's idea. Collagenous materials and ceramics are commercially available for medical use. Collagenous materials are a single component (mainly type I collagen) or a mix of type I collagen and gelatin. The collagen fibers are derived from bovine or pig. Hydroxyapatite (**Figure 3**), carbonate apatite, and β -TCP are bio-ceramics without growth factors and organic matter (**Table 1**).

10. Clinical case with DDM scaffolds for onlay graft on atrophic mandible

10.1 Patient

A 63-year-old female presented with missing teeth (#35-#37). Root apical lesion was found in #34, and periodontitis was observed in #14 and 15 regions (**Figure 5a**). A clinical examination revealed highly atrophic bone in the mandible. Her medical history was unremarkable.

10.2 Surgical procedure 1

X-ray photos were taken before surgery. Non-vital teeth with lesion (#14, 15, 34) were extracted, and the debris and foreign body were removed carefully. Two teeth were prepared for root-type DDM (**Figure 5b**). The roots were perforated by a round bur, using the medical device (FIX®, Tokyo Iken Co., Ltd), and demineralized in 2% HNO_3 solution for 30 min. The other was crushed with saline ice by an electric mill

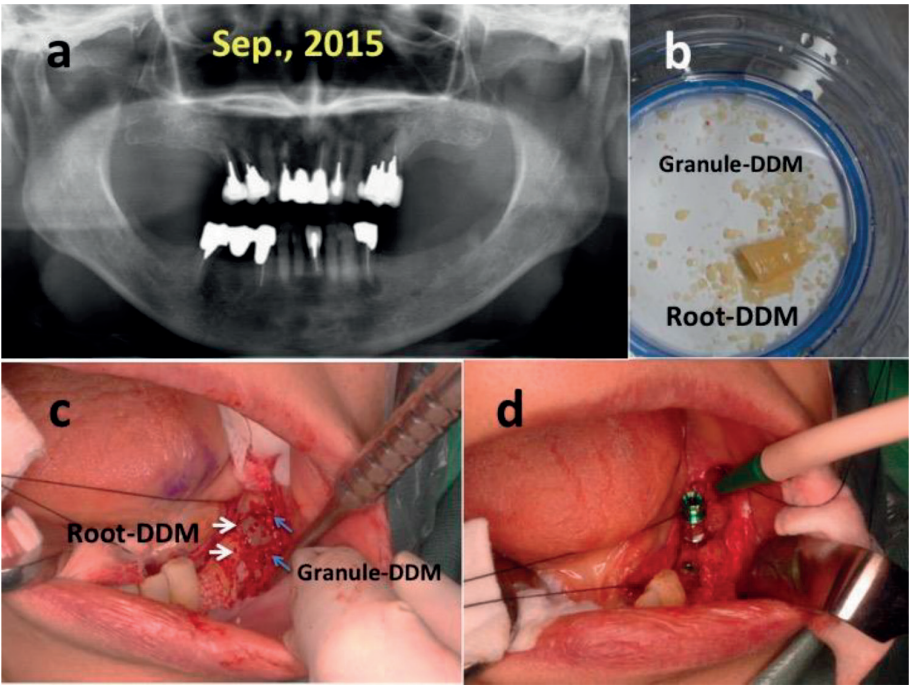


Figure 5.
Case: 63-year-old woman, lateral augmentation (#35 and 36 regions) by DDM. (a) Initial X-ray view; (b) DDM materials (roots, granules); (c) immediate DDM graft; note, lateral augmentation; (d) view after placement of fixtures.

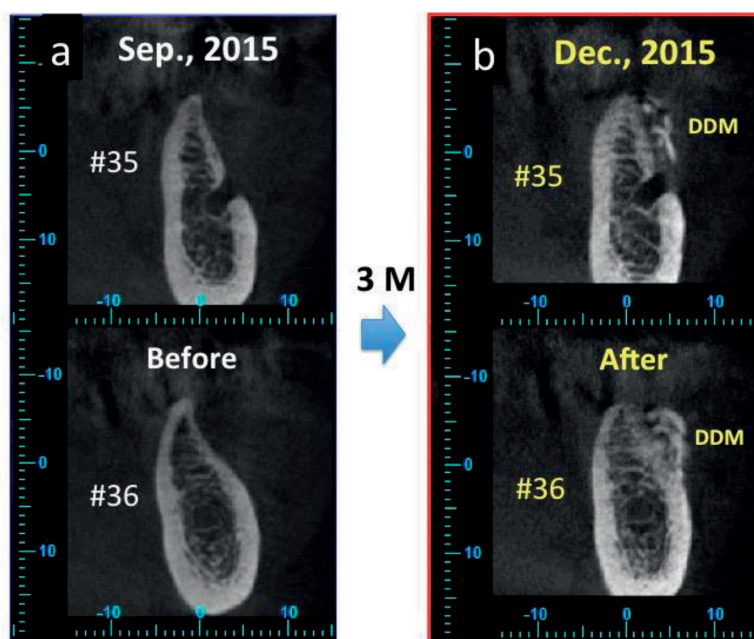


Figure 6.
X-ray CT before and after augmentation by DDM in 63-year-old woman. (a) Initial X-ray CT (#35, 36 region); note, ridge of atrophic bone; (b) 3 months after DDM graft; note, DDM combined with original bone.

(Osteo-Mill®, Tokyo Iken Co., Ltd) at 12,000 rpm for 30 s (Japan Patent: 4,953,276; USA Patent: 8,752,777). Next, the crushed tooth granules were decalcified in 2% HNO₃ for 20 min [55]. Two types of DDM were rinsed in cold distilled water. Perforations were performed into the atrophic bone (#35, 36) [43, 56]. DDM scaffolds were grafted for lateral augmentation (**Figure 5c**), covered by collagen membrane, and immediate implant placement (Ø3.7 mm) was done into #34 socket under intravenous sedation.

10.3 Surgical procedure 2

X-ray CT was taken at 3 months after DDM graft. DDM residues did not get out during the drilling, and two fixtures (Ø3.7 mm) were implanted into the augmented bone (#35, 36) (**Figure 5d**).

10.4 Results and discussion

The CT images at 3 months showed remarkable lateral augmentation, compared with that of preoperation (**Figure 6**). Non-vital teeth-derived DDM scaffolds were received to host, and two fixtures (Ø3.7 mm) were placed into the DDM-augmented bone. Partially demineralized dentin matrix from non-vital tooth is acellular composite of collagen and hydroxyapatite without BMP activity. As original bone contains BMP activity, bone managements such as ultrasonic scaler treatment and perforations into cortical bone must be essential.

This patient was successfully restored with her own teeth and implant surgery. This case was a unique DDM onlay graft, combined with two types of DDM (roots, granules) for lateral bone augmentation in 2015.

11. Conclusion

Vital- or non-vital tooth-derived DDM can be recycled as natural scaffolds for local bone engineering. DDM graft is a matrix-based therapy without cells. Doctors can use patient's own teeth as bone graft materials. Biomaterial science should

support and develop the advanced regenerative therapy using tooth-derived materials for patients in the near future.

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