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Autograft of Dentin Materials for Bone Regeneration

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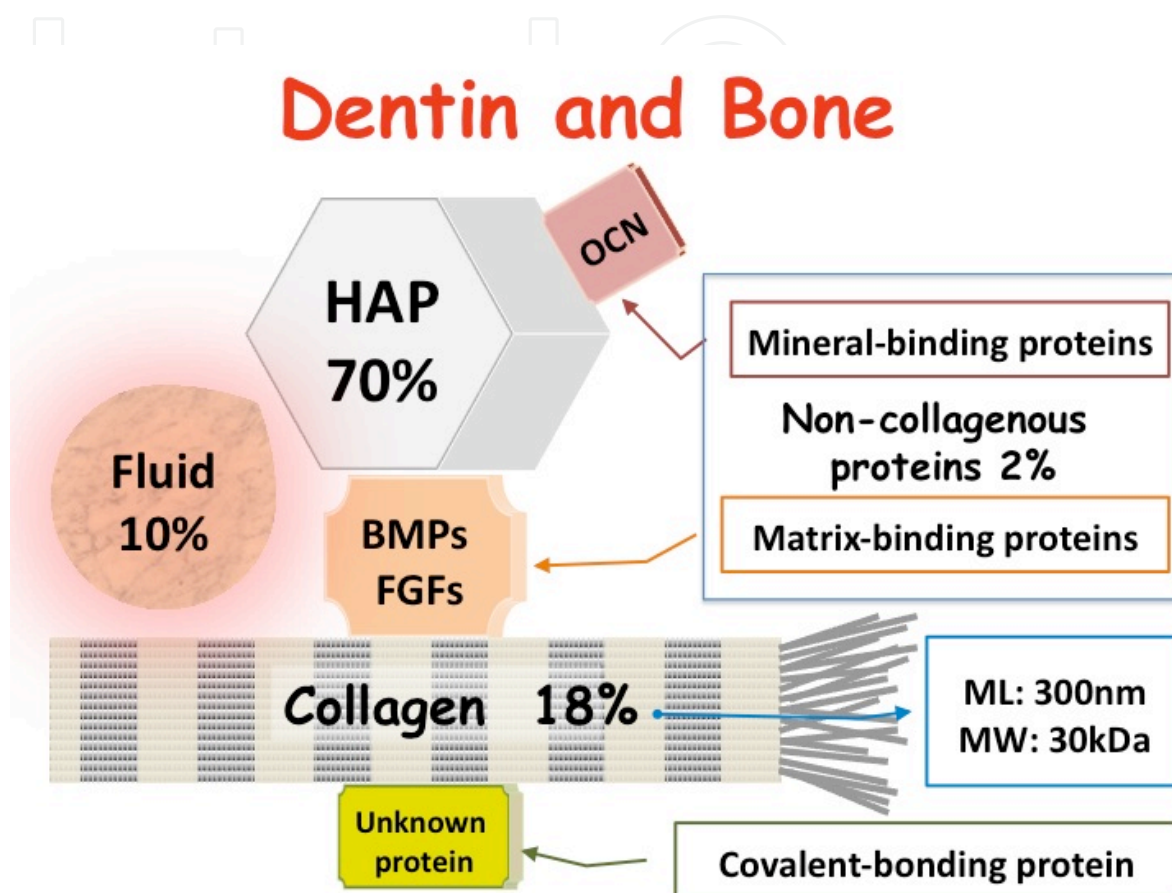
1. Introduction

Regenerative medicine is based on advanced and applied biomaterials science. Biomaterials have a major impact on the patient cure for improving the quality of life. We have been challenging to develop bioabsorbable dentin materials (Murata et al, 2011; Murata et al, 2012), harmonized with bone remodelling, by using the supersonic and acid-etching technology (Akazawa et al, 2012).

While human bone autograft was done in 19th century, human dentin autograft for bone augmentation was reported in IADR 2003. The first clinical case was a sinus lifting using auto-dentin for bone augmentation (Murata, 2003). Dentin is acellular matrix, while bone include osteocytes. Very interestingly, biochemical components in dentin and bone are almost similar. They consist of body fluid (10%), collagen (18%), non-collagenous proteins (NCPs: 2%) and hydroxyapatite (HAp: 70%) in weight volume (Fig. 1). Demineralized dentin matrix (DDM) and demineralized bone matrix (DBM) are mainly type I collagen with growth factors such as bone morphogenetic proteins (BMPs) (Urist, 1965) and fibroblast growth factors (FGFs) (Fig. 2) (Butler et al.,1977; Murata et al, 2010a,b).

Korea Tooth Bank (KTB) was established in Seoul 2009 for an unique service of tooth-derived graft materials. The medical service system is the preparation and delivery of the tooth-derived materials on demand (Kim et al,2010; Kim et al, 2012). The tooth-derived materials were named as auto-tooth graft materials, which divided into the block-type and powder-type (Park et al., 2010). The block-type material, which is hydrated in 0.9% NaCl solution for 15-30 min before use, can be cut by operators with surgical knife or scissors. Recently, the enamel-dentin

grafting has been becoming a realistic alternative to the bone grafting in Korea. We have thought the non-functional teeth as native resources of various graft materials and have achieved the medical recycle of patient-own teeth as novel materials for bone regeneration in Japan and Korea. This matrix-based bone therapy is *Dental Innovation* early in 21st century. Our innovative technique will expand from East Asia to the world.



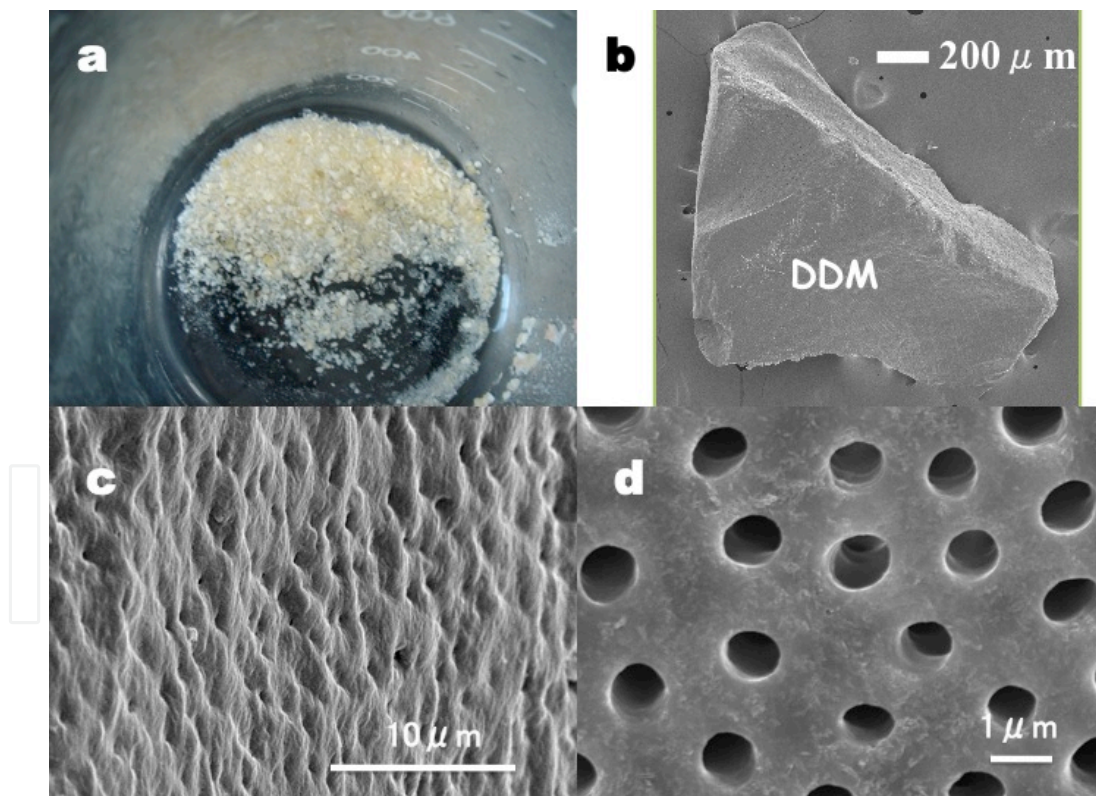
BMPs, FGFs: matrix-binding proteins in NCPs. OCN: mineral-binding proteins in NCPs; Collagen: mainly type I collagen

Figure 1. Chemical components (w/v%) of human dentin and bone;

2. Biochemistry of human dentin

Dentin and bone are mineralized tissues and almost similar in chemical components. They consist of body fluid, collagen, non-collagenous proteins (NCPs) and hydroxyapatite (HAp) in weight volume (Fig. 1). The NCPs in dentin and bone are secreted into the ECM in the process of biomineralization. The category is termed the SIBLING (Small Integrin-Binding Ligand, N-linked Glycoprotein) family that includes dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP) and osteopontin (OPN) (Fisher et al, 2001; Qin et al, 2007; Sun et al, 2010; Qin et al, 2011).

Both DDM and DBM are composed of predominantly type I collagen (95%) and matrix-binding proteins such as BMPs (Murata et al., 2000; Akazawa et al., 2006; Murata et al., 2007). BMPs, transforming growth factor-beta (TGF- β), insulin growth factor-I (IGF-I) and IGF-II were detected in human dentin (Finkelman et al., 1990). In the rabbit study, completely demineralized dentin matrix induced bone in the muscle at 4 weeks, while calcified dentin induced bone at 8-12 weeks after implantation (Yeoman & Urist, 1967; Bang & Urist, 1967). Many researchers made effort to discover dentin-derived BMPs. (Butler et al., 1977; Urist et al., 1982; Kawai & Urist., 1989; Bessho et al., 1990). In our study, human DDM and human DBM induced bone and cartilage independently in the subcutaneous tissues at 4 weeks (Murata et al, 2010b). These results indicated that highly calcified tissues such as cortical bone and calcified dentin are not earlier in osteoinduction and osteoconduction than spongy bone, DBM, and DDM. The delayed inductive properties of the calcified dentin and bone may be related to the inhibition of BMPs-release by HAp crystals (Huggins et al., 1970).



a: wet granules, b,c,d: SEM of DDM granule. Note: dentinal tubes

Figure 2. Crushed tooth granules and SEM photos of demineralized dentin matrix (DDM)

DDM is defined as an acid-insoluble dentin collagen that is absorbable, but hard to digest in human body (Fig. 2). DDM is acellular biomatrix with the micro-tube structure. DDM and DBM possess the ability to coagulate blood plasmas (Huggins & Reddi., 1973). The coagulation action of blood plasma by DDM should become advantageous for surgical operations.

Dentin formation is a dynamic and complicated process, involving interplays among a number of molecules including type I collagen, NCPs and proteoglycans, which work collectively to precisely control the site and rate of apatite formation. Type I collagen secreted by odontoblasts forms the scaffold, upon which HAp crystals are deposited. In addition to type I collagen, the extracellular matrix contains a number of NCPs which play critical roles in the initiation and regulation of HAp crystals (Qin et al., 2011).

3. Clinical study of human dentin

3.1. Case 1: Bone augmentation, 17 year-old female

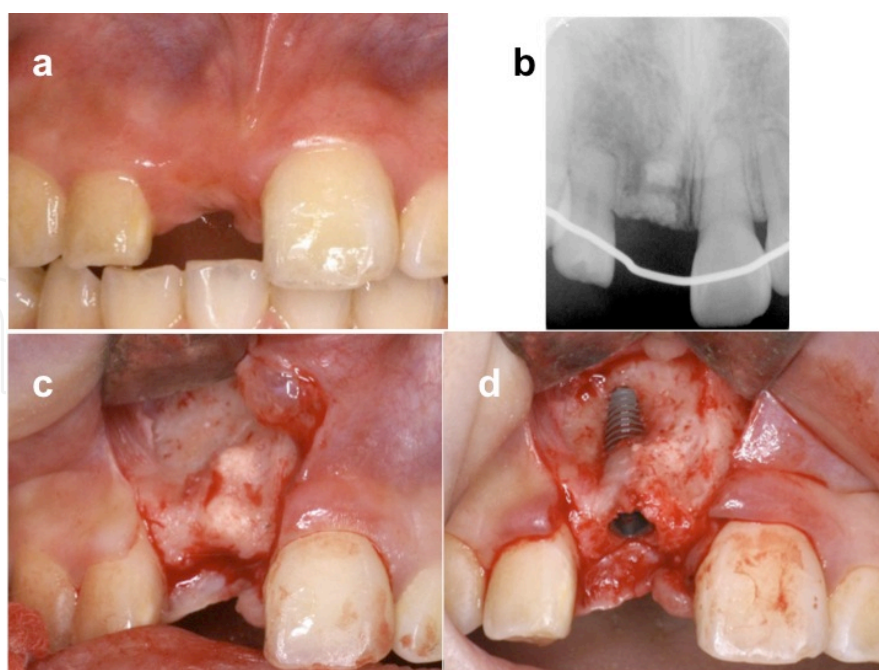
Patient: A 17-year-old female presented with missing teeth (#11). Clinical and radiological examinations revealed atrophied bone and fractured root residue in the region (Fig. 3a,b). Her medical history was unremarkable.

Surgical procedure 1: Four wisdom teeth were extracted for the preparation of tooth-derived materials (block-type, powder-type).

Preparations of dentin materials: The extracted molar was divided into the crown portion and the root portion. The crown portion was crushed under the cooling. The crushed granules were decalcified in 0.6N HCl solution, rinsed in cold distilled water and freeze-dried. On the other hand, the root portion was perforated by using a round bar to create a porous structure. The root with many holes was decalcified in 0.6N HCl solution, rinsed and freeze-dried. These biomaterials are named as auto-tooth bone (ATB) by KTB.

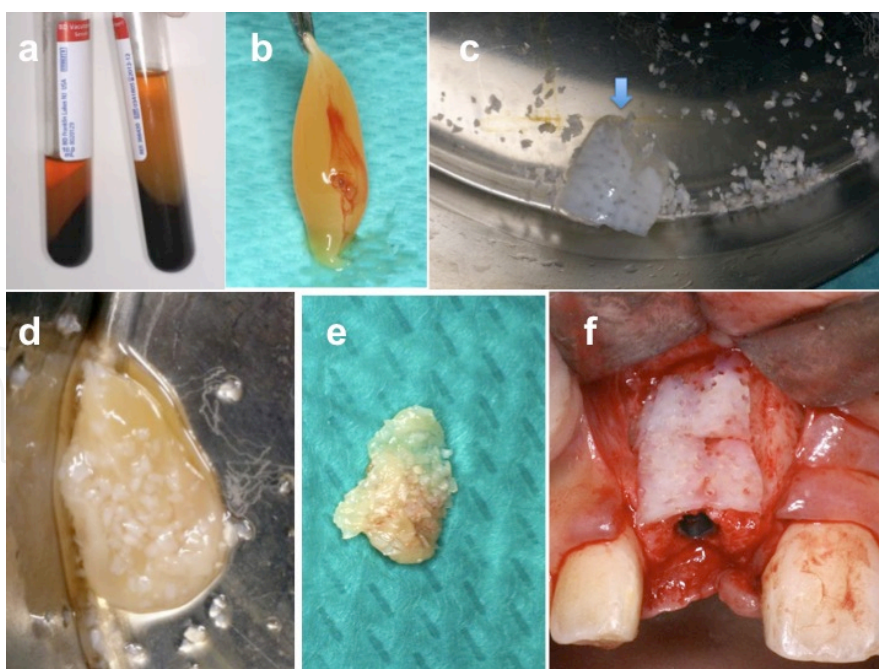
Surgical procedure 2: This patient-own blood sample was centrifuged and the middle layer was collected as fibrin glue (so called concentrated growth factors: CGF) (Fig. 4a,b). The different ATB materials were immersed in 0.9% NaCl solution before use (Fig. 4c). Additionally, ATB granules were mixed with the fibrin glue (CGF) prepared from autologous blood (Fig. 4d,e). The root-dentin material was divided into 2 parts by using a knife. A titanium fixture (Nobel Replace Tapered NP: 16mm) was implanted into the atrophied bone under local anesthesia (Fig. 3c,d). The root-dentin wall was grafted into the bone defect (fixture-exposed region) as veneer graft (Fig. 4f). The composite of ATB and fibrin contributed to the attachment between the grafted root-dentin and the muco-periosteal flap (Fig. 5a,b,c).

Results and discussion: This patient was successfully restored with the dental implant and the autograft of 2 types of ATB (root-on, powders) with autologous fibrin glue (Fig. 5d). Properly hydrated ATB should facilitate its adaption to the bone defect due to its elasticity and flexibility. The results demonstrated that autogenous tooth could be recycled as the innovative biomaterials.



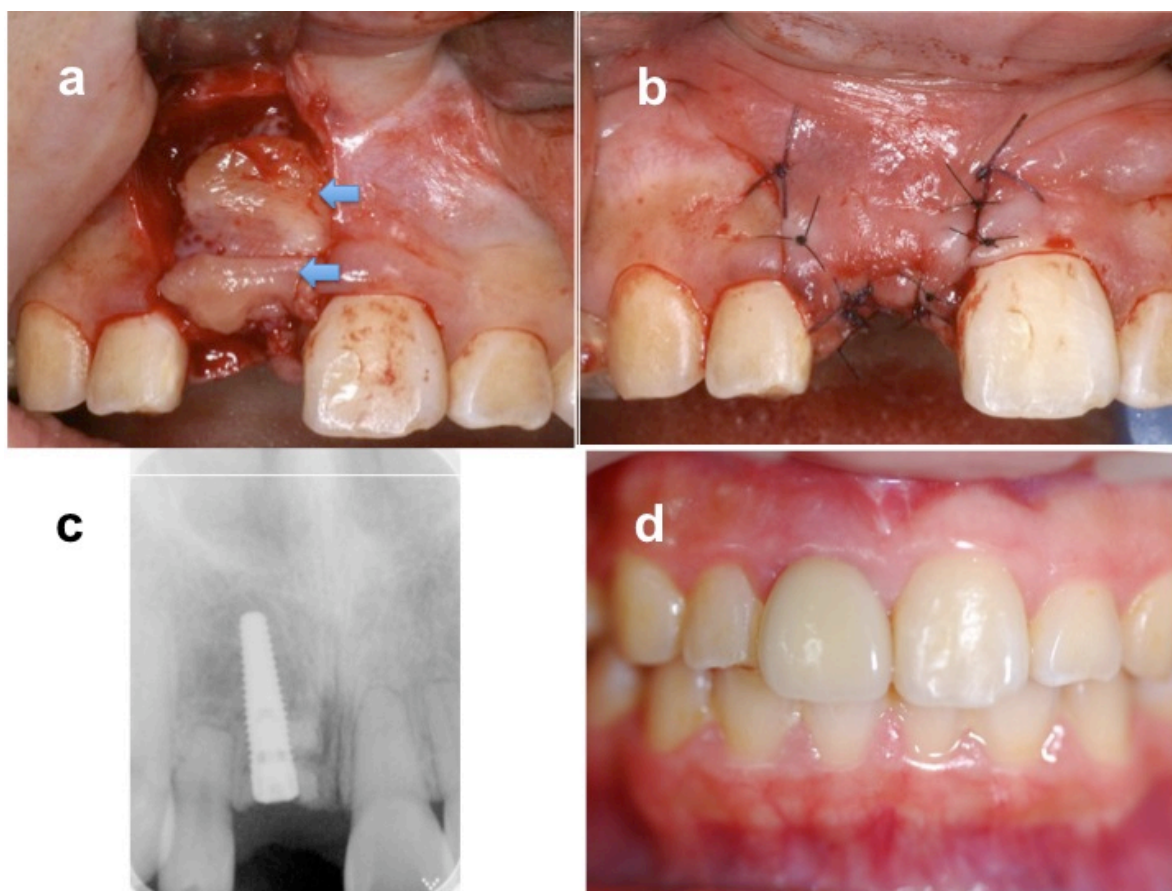
a: intraoral initial view (before operation), Note: a missing tooth (#11) b: X-ray photo, Note: radio-opacity of residual root c: exposed bone, Note: concave shape d: view just after Ti. fixture implantation, Note: labial bone defect

Figure 3. Case 1: Auto-tooth bone graft for implant placement, 17 year-old girl;



a: blood after centrifugation, Note: 3 layers b: fibrin glue; middle layer in 4a c: wettable ATB materials (block-type ↓, powder-type) d,e: composite of powder and fibrin glue f: covering with block-type of dentin

Figure 4. Case 1: Auto-tooth bone (ATB) graft for implant placement, 17 year-old girl



a: fibrin glue including ATB powders (⇔) b: repositioned flap. Note: suture with nylon c: X-ray photo just after operation d: final view after prosthetic restoration

Figure 5. Case 1: Auto-tooth bone graft for implant placement, 17 year-old girl

3.2. Case 2: DDM onlay graft and tooth autograft, 25 year-old female

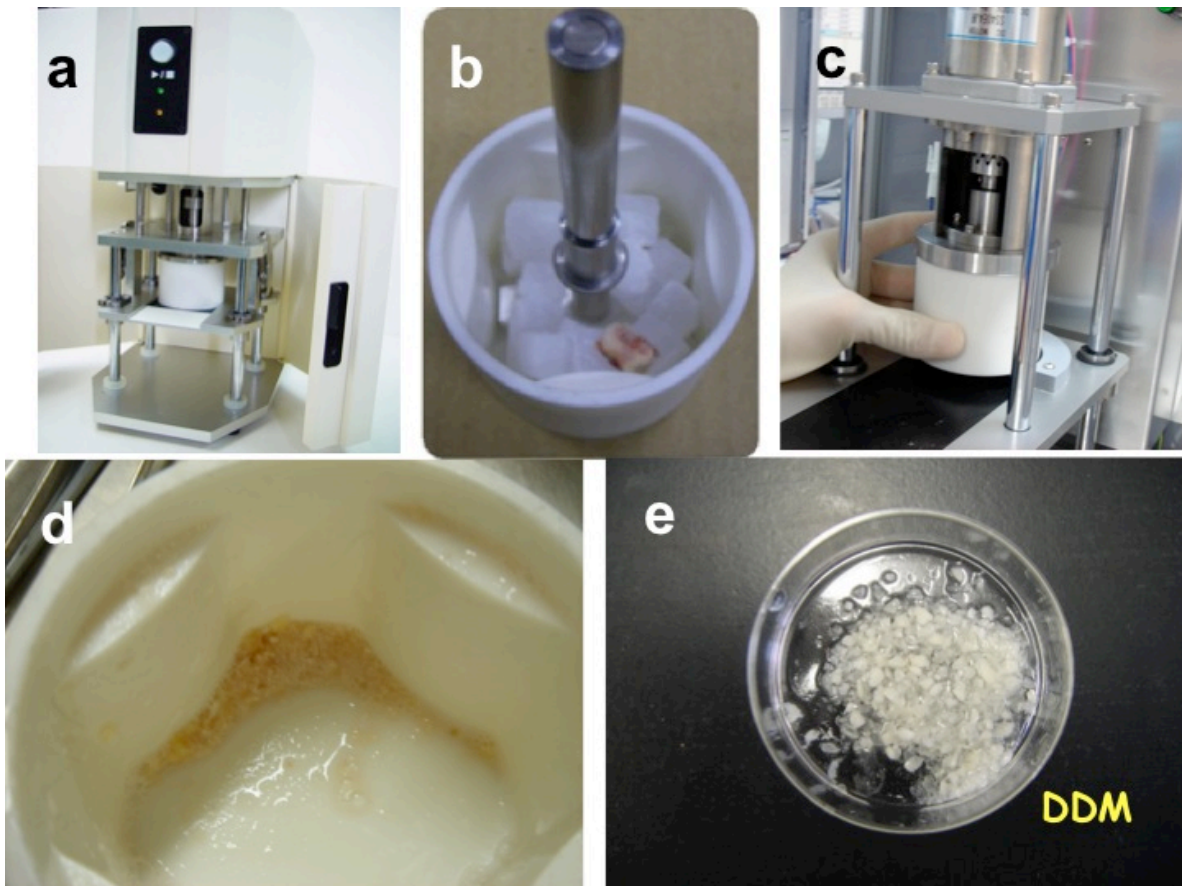
Patient: A 25-year-old female presented with missing teeth (#46). She lost the first molar about 12 years ago. A clinical examination revealed an atrophied bone in the region. Her medical history was unremarkable.

Surgical procedure 1: A non-functional vital tooth (#28) was extracted and immediately crushed with saline ice by our newly developed tooth-mill (Osteo-Mill®, Tokyo Iken Co., Ltd) at 12000rpm for 30 sec (Fig. 6) (Patent: 4953276). Briefly, vessel and blade were made in ZrO_2 . The crushed tooth-granules were decalcified in 2% HNO_3 solution for 20 min (Murata et al., 2009). The DDM granules including cementum were rinsed in cold distilled water. Cortical perforations were performed in the atrophied bone, and DDM were immediately autotransplanted on the perforated bone under local anesthesia.

Surgical procedure 2: At 4 months after the first operation, a non-functional vital tooth (#18) was extracted and received the immediate root canal filling (RCF), using a new fixation device (Fig. 7). The device was developed for tooth transplantation and replantation (Patent: 4866994).

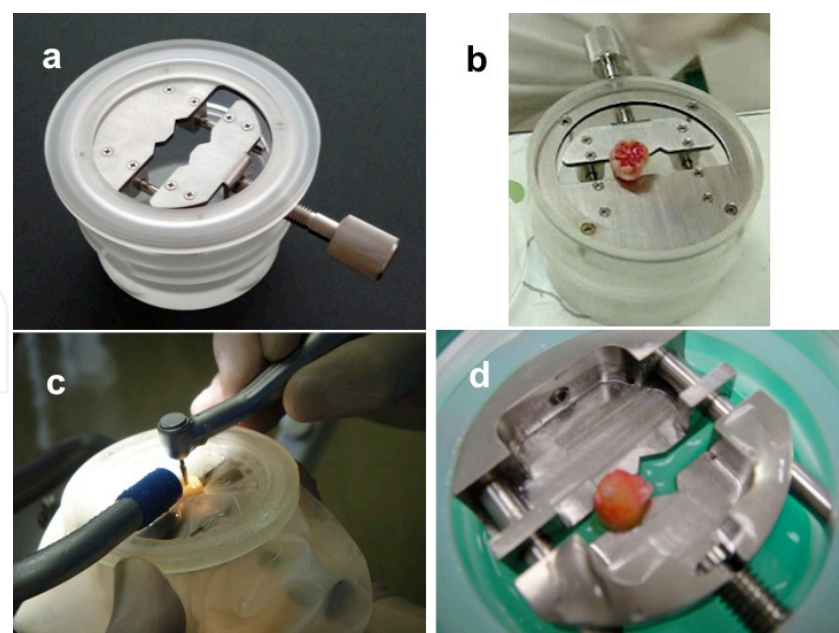
After the bone biopsy for the tissue observation and the preparation of transplanted cavity, tooth autograft was carried out into the host bone (Fig. 8a,b,d).

Results and discussion: The biopsy tissue showed that DDM granules were received to host, and partially replaced by new bone (Fig. 8e). This case was onlay graft of DDM on perforated cortical bone (Murata et al, 1999; Murata et al, 2000). Though RCF is generally carried out at more than 4 weeks after tooth transplantation, we did immediate RCF, using the medical device. This patient was successfully restored with her own 2 teeth. This case was the immediate tooth autotransplantation with the immediate root canal filling at 4 months after DDM autograft in 2009.



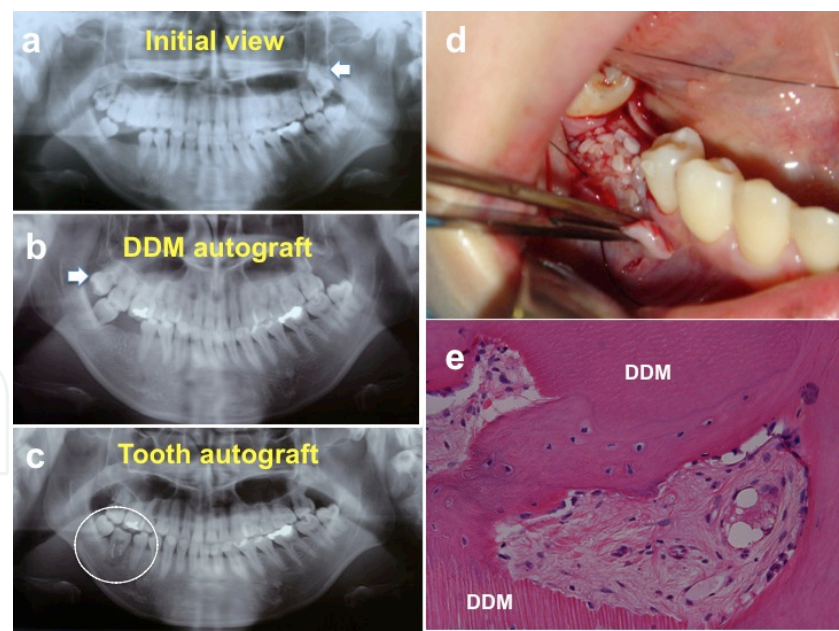
a: mill, b: tooth with ice blocks, c: ZrO₂ vessel, d: crushed tooth, e: DDM granules before clinical use.

Figure 6. Preparation of DDM using automatic tooth mill (Osteo-Mill®, Tokyo Iken)



a: whole view, Note: the device developed for tooth transplantation and replantation b: fixed tooth, Note: correspondence to all teeth c: crown treatment, Note: periodontal ligament tissue protected from infected fine particles d: root view, Note: keeping blood even after cutting and root canal filling

Figure 7. New device for protecting periodontal ligament cells (Mr.FIX®, Tokyo Iken)



a: initial X-ray photo: missing tooth (#46) and atrophied bone. Non-functional tooth (⇔) for DDM b: just after DDM graft. Non-functional tooth (⇔) for next tooth autograft c: tooth auto-transplantation at 4 months after DDM graft d: DDM autograft on perforated cortical bone before suture e: biopsy: mature bone connected with DDM residue (HE section)

Figure 8. Case 2: 24 year-old woman

4. Supersonic and acid-etching method for Dentin geometry

Compact structure inhibits the body fluid permeation and the cell invasion into the inside of the materials. Generally, this situation is called a material wall. Dentin and cortical bone have compact structure. We have been challenging to develop new dentin materials, using a supersonic and acid-etching technology (Akazawa et al., 2009; Akazawa et al., 2010; Akazawa et al., 2012). The surface structure design of dentin by the supersonic treatment might easily produce new functional scaffolds, which control the bio-absorption rate and the adsorption ability for protein and cells. Figure 9 shows the dissolution efficiencies of human dentin granules, which were demineralized for 5-45 min in 2.0%-HNO₃ solutions by the supersonic treatment at 600W. A photograph inside Fig.9 is Digital microscopic view of DDM, dissolution for 30 min in 2.0%- HNO₃ by the supersonic treatment at 600W and 28 kHz.

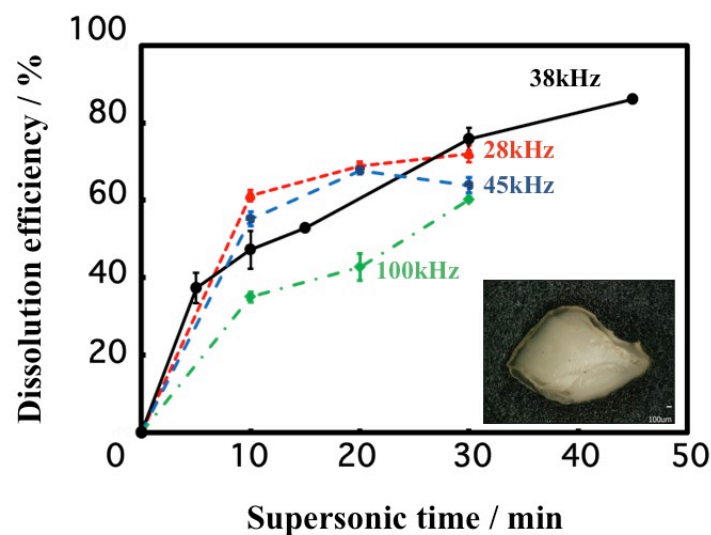


Figure 9. Dissolution efficiencies of human dentin granules, demineralized for 5-45 min in 2.0%-HNO₃ by supersonic treatment at 600W. Inside photo: Digital microscopic DDM view, dissolution for 30 min in 2.0%- HNO₃ by supersonic treatment at 600W and 28 kHz.

The innovative technology can create the adequate geometry and the surface structure of commercially available materials (Akazawa et al., 2012). Geometrical factors will improve the performance of biomaterials for bone regeneration (Reddi, 1974; Kuboki et al, 1995; Murata et al, 1998). Biomaterials science should support and develop the advanced regenerative therapy using tooth-derived materials for patients in the near future.

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