A New Method for Alveolar Bone Repair Using Extracted Teeth for the Graft Material

Tomoki Nampo,* Junichi Watahiki,* Akiko Enomoto,* Tomohiro Taguchi,* Miki Ono,* Haruhisa Nakano,* Gou Yamamoto,† Tarou Irie,† Tetsuhiko Tachikawa,† and Koutaro Maki†

Background: In the clinical field of jawbone formation, the use of autogenous bone as the graft material is the gold standard. However, there are some problems with this technique, such as risk of infection on the donor side, the limited amount of available bone mass, and marked resorption of the grafted bone. We investigated the potential for using teeth as a bone graft material for jawbone formation because the dental pulp contains stem cells, including undifferentiated neural crest–derived cells.

Methods: Alveolar bone defects were created in Wistar rats, and the defects were filled with either tooth or iliac bone graft material, or left as controls. The potential for using teeth as a bone graft material for jawbone formation was measured using real-time polymerase chain reaction, microcomputed tomography, and histologic analysis.

Results: Polymerase chain reaction revealed that the expressions of P75, P0, nestin, and musashi-1 were significantly higher in teeth than in mandibular bone and iliac bone grafts. Hematoxylin and eosin staining and microcomputed tomography showed that at 8 weeks, tooth graft material produced a similar amount of new bone compared to iliac bone graft material. Osteopontin was expressed in both the tooth and iliac bone graft material at 6 and 8 weeks after surgery. Dentin sialoprotein was expressed in the tooth graft material in the new bone at 6 weeks only.

Conclusion: These results indicate that teeth may be an alternative material to autogenous bone for treating alveolar bone defects by grafting. *J Periodontol 2010;81:1264-1272.*

KEY WORDS

Bone regeneration; bone substitute; grafts, bone; neural crest; tooth.

* Department of Orthodontics, School of Dentistry, Showa University, Tokyo, Japan.
† Department of Oral Pathology and Diagnosis, School of Dentistry, Showa University.

In the field of clinical dental bone formation, various bone graft materials are used. These include allografts (e.g., demineralized freeze-dried bone allografts and freeze-dried bone allografts); xenografts, (e.g., bovine bone and coral); and alloplasts, (e.g., ceramics for biologic use, β-tricalcium phosphate [β-TCP] and hydroxyapatite). Three properties are required for an ideal bone graft material: 1) osteoconduction, which provides scaffolds for bone regeneration;[1] 2) osteoinduction, which promotes the recruitment of bone-forming cells, such as undifferentiated cells and preosteoblasts, and formation of bone from these cells;[1,2] and 3) osteoproliferation, the induction of cells contained in the graft material to promote bone regeneration.[3] Allografts lack osteoproliferation, and xenografts and alloplasts only show osteoconduction. Because only autogenous bone exhibits all three properties, autogenous bone grafting is currently considered the best method.[4] The iliac bone is a frequently used autogenous bone and it is grafted into alveolar bone defects in most cases of cleft palate.[5] However, there are problems with autogenous bone grafting, such as risk of infection at the donor side, limited amount of available bone mass, and marked resorption of the grafted bone.[6,7]

Developmentally, most bones of the trunk and extremities, including the iliac bone, are formed by endochondral

doi: 10.1902/jop.2010.100016

ossification, whereas the jaw and alveolar bones are formed by intramembranous ossification.[8] Using bone with a different ossification pattern from those of the jaw and alveolar bones as a graft material for jawbone reconstruction is a matter of concern. Donovan et al.[9] and Donos et al.[10] grafted iliac and cranial bones and investigated the graft bone resorption rate after 6 months. They observed that about twice as much iliac bone was resorbed compared with grafted cranial bone. Jaw and alveolar bones both differentiate from neural crest cells.

Vertebrates develop from three germ layers, ectoderm, mesoderm, and endoderm, and a type of tissue originating from neural tube fusion region, neural crest cells. This is called the fourth germ layer because of its importance. Neural crest–derived cells have been shown to exhibit multipotency and differentiate into mesodermal mesenchymal cells, despite being ectodermal. They show a high capacity for self-regeneration and persist in adult tissues.[11,12]

Tissues derived from the neural crest include the maxillofacial bones (excluding the occipital, sphenoid, temporal, and ethmoid bones); cartilage; teeth; and nerve and glial cells.[13,14] Of these, teeth contain stem cells in the dental pulp, and it has been suggested that the dental pulp contains undifferentiated neural crest–derived cells.[15-17] Seo et al.[18] cultured stem cells isolated from dental pulp, grafted them into a defect prepared in the cranial bone, and observed hard tissue formation. In addition, dentin contains growth factors: insulin-like growth factor (IGF)-II, bone morphogenetic protein (BMP)-2, and transforming growth factor (TGF)-β.[19] Cementum contains TGF-β, IGF-I, and type I and III collagen.[20] Saygin et al.[21] suggested that the use of cementoblasts for periodontal tissue regeneration is worthwhile. Isaka et al.[22] reported that the periodontal ligament has the ability to regenerate bone, and Flores et al.[23] regenerated periodontal tissue using cultured periodontal ligament cells. The periodontal ligament also contains TGF-β, IGF-I, basic fibroblast growth factor, vascular endothelial growth factor, BMP-2, platelet-derived growth factor (PDGF), and type I collagen.[24] Furthermore, dentin and cementum contain proteins common to bone, such as osteopontin (OPN), bone sialoprotein (BSP), osteocalcin, dentin sialoprotein (DSP), dentin matrix protein-1 (DMP-1), type I collagen, osterix, and Cbfa1 (Runx2). These are reportedly involved in bone formation and resorption.[25,26]

Thus, we considered that teeth containing undifferentiated neural crest–derived cells, proteins involved in bone formation, and growth factors may be used as a bone graft material for jawbone formation.

Although there has been no study in which teeth were used as a bone graft material, tooth replacement by bone has been reported. In this previous case, osteoclast cells appeared in the pulp cavity after tooth reimplantation and the pulp was replaced by bone tissue, followed by root resorption and ankylosis. Finally, the whole root was integrated into the surrounding alveolar bone.[27-29] These reports show that the jawbone and teeth have a high level of affinity for each other.

Based on the previous information, we investigated the possibility of using teeth as a bone graft material for jawbone formation by comparing it to autogenous iliac bone grafts.

MATERIALS AND METHODS

Real-Time Polymerase Chain Reaction (PCR)

RNA isolation. Tooth, iliac bone, and mandibular bone (control) were extirpated from 12-week-old male Wistar rats (350 to 400 g), and RNA was extracted from the tissue exposed to the RNA stabilizing treatment‡ according to the manufacturer's protocol.

Reverse transcription. After the isolation of the RNA, a reverse transcriptase (RT) kit§ was used to make cDNA. The mixture was composed of 13 μl of total RNA, 2 μl of random primers, 2 μl of deoxynucleotide triphosphate, 2 μl of buffer RT, and 1 μl of omniscript RT added to a final volume of 20 μl. The mixture was heated‖ for 60 minutes at 37°C.

Semiquantitative PCR. The cDNA from the RT reaction was used as a template in the PCR. We used four primers (P75,¶ P0,# nestin,** and musashi-1††). The PCR mixture was composed of 2 μl of sample cDNA, 1 μl of each primer, 7 μl of RNase-free water, and 10 μl of a gene expression mix‡‡ for to a final volume of 20 μl. We performed real-time PCR§§ for 50 cycles at 95°C for 15 seconds, 60°C for 1 minute followed by 50°C for 2 minutes, and 95°C for 10 minutes. We calculated the relative expression level by dividing the signal intensity of each gene by that of GAPDH.‖‖ For quantification, a series of five-fold dilution standards and a negative control (RNase-free water) were run alongside the samples.¶¶

Animals

Sixty 12-week-old male Wistar rats were randomly divided into three groups of 20. Ten rats in each group were sacrificed at 6 weeks, and the remaining 10 at 8 weeks. The groups were as follows: group 1 (tooth

‡ RNAlater, Qiagen, Hilden, Germany.
§ Omniscript Reverse Transcriptase Kit, Qiagen.
‖ 2400 GeneAmp PCR System, PerkinElmer Japan, Tokyo, Japan.
¶ Rn00586061_s1, Applied Biosystems, Foster City, CA.
# Rn00566746_m1, Applied Biosystems.
** Rn00564394_m1, Applied Biosystems.
†† Rn00596059_m1, Applied Biosystems.
‡‡ TaqMan Gene Expression Master Mix, Applied Biosystems.
§§ 7500 ABI PRISM, Applied Biosystems.
‖‖ Rn99999916_s1, Applied Biosystems.
¶¶ Applied Biosystems.

group), tooth except enamel with β-TCP[##] complex; group 2 (bone group), iliac bone with β-TCP complex (positive control); and group 3 (control group), no material (negative control). The Animal Research Committee of Showa University, Tokyo, Japan, approved all procedures.

Graft Material Preparation

All surgical procedures were performed under general anesthesia in sterile conditions. After inhalation of anesthesia with ethyl ether,[***] general anesthesia was achieved with an intraperitoneal injection of pentobarbital sodium.[†††] In the tooth group, a tooth was extracted on the side opposite to the area where the alveolar bone defect was made. The crown portions of the extracted teeth were removed with scissors, and the root portions of the remaining teeth were trimmed as closely as possible to 500 μm at once. Next, the trimmed tooth was mixed with a measured quantity of β-TCP. These grafts were prepared on ice. In the bone groups, the cancellous bone of the iliac bone was removed, granulated, and mixed with β-TCP. The graft material used in both groups was approximately 0.2 g. The ratio of the mixture of the transplant material and β-TCP was adjusted to 2:1.

Surgical Protocol

An incision was made in the palate, and a full-thickness flap was created exposing the alveolar bone. An alveolar bone defect, 2 mm in diameter, was made with a diamond bur. Subsequently, one of the two materials was grafted into each alveolar bone defect (the control group did not receive implantation of a graft material), and a resorbable bilayer collagen membrane[‡‡‡] was placed over the bone defect in all the groups. All the graft materials were transplanted to the defect within 30 minutes after extirpation. The flap was repositioned and sutured tightly with resorbable sutures,[§§§] covering the bone defect.

Post-surgical Care

All the rats received antibiotics (penicillin G potassium, 200,000 units[‖‖‖]) intramuscularly daily for 3 days after surgery. The rats were fed a soft diet[¶¶¶] for 2 weeks to reduce any potential mechanical damage.

Microcomputed Tomography (μ-CT) Observations

μ-CT[###] was used to observe new bone formation. Images were acquired immediately after surgery and at 6 and 8 weeks. We confirmed the maxillary-bone form of an intact rat by a pilot experiment.

Histologic Procedures

Animals were euthanized 6 and 8 weeks after surgery with an overdose of ethyl ether. All defects in the groups were dissected along with the surrounding soft and hard tissues. Block sections were fixed with 4% paraformaldehyde, decalcified[****] for 2 days, neutralized with 5% sodium sulfate anhydrous, and then embedded in paraffin. Sections were cut, deparaffinized, and stained with hematoxylin and eosin (H&E). We confirmed the maxillary-bone form of an intact rat by a pilot experiment in the tissue section.

Immunohistochemical Procedures

Sections (7 μm) were cut, mounted on slides, deparaffinized, and incubated with anti-OPN antibody[††††] and anti-DSP antibody[‡‡‡‡] at appropriate dilutions. Specimens to be reacted with anti-DSP antibody were pretreated by using the activator[§§§§] for 10 minutes.

RESULTS

Real-Time PCR

Real-time PCR results for the expressions of P75, P0, nestin, and musashi-1 in the tooth, iliac bone, and mandibular bone (control) were quantitatively determined (Fig. 1). The expressions of all four genes were significantly higher in the tooth than in the mandibular bone and iliac bone graft material. In the iliac bone, the expressions of all four genes were insignificant.

Clinical Observations

Wound healing was uneventful. Six weeks after surgery, the wounds of the tooth, bone, and control groups appeared to be similar. No material exposure or intense inflammatory reactions were observed during the healing period.

μ-CT Observations

The absorption of grafted materials and the formation of new bone with time in both the tooth and bone groups were confirmed with μ-CT (Figs. 2 and 3). In the bone group, new bone formation exceeding the defective bone region was noted 6 weeks after surgery, but marked resorption of the new bone occurred at 8 weeks. In the tooth group, new bone formation filled the bone defect at 6 weeks, and the new bone was retained at 8 weeks. Little new bone formation was noted in the control group at 8 weeks.

Histologic Observations

In the tooth group, slight inflammatory reactions were noted around the defects at 6 weeks, but new bone formation was confirmed in the defects. In the bone

Curasan, Kleinostheim, Germany.
*** Wako Pure Chemical Industries, Osaka, Japan.
††† Kyoritsu Seiyaku Corporation, Tokyo, Japan.
‡‡‡ Geistlich Pharma.
§§§ BEAR Medic Corporation, Ibaraki, Japan.
‖‖‖ Meiji Seika Kaisha, Tokyo, Japan.
¶¶¶ Nihon Nosan Kogyo Corporation, Yokohama, Japan.
eXplore Locus CT System and MicroView, GE Healthcare, Tokyo, Japan.
**** KALKITOX, Wako Pure Chemical Industries.
†††† Cosmo Bio, Tokyo, Japan.
‡‡‡‡ Santa Cruz Biotechnology, Santa Cruz, CA.
§§§§ Gold Standard Series L.A.B. Solution, Polysciences, Warrington, PA.

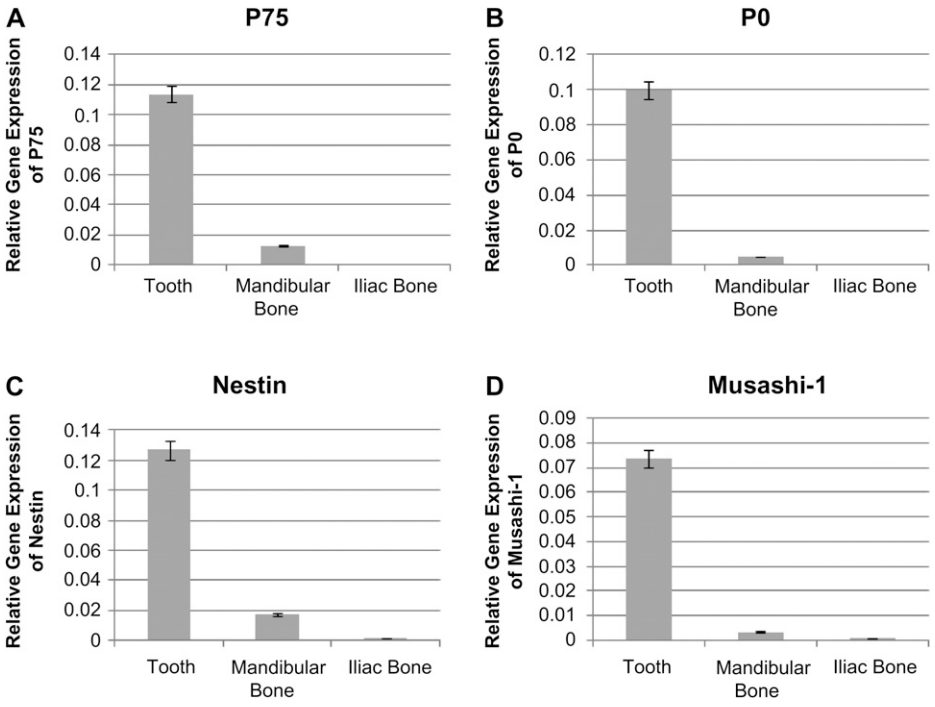

Figure 1.
Expressions of P75 ***(A)****, P0* ***(B)****, Nestin* ***(C)****, and Musashi-1* ***(D)*** *in tooth, iliac bone, and ungrafted control (mandibular bone). Twenty 1 2-week-old male Wistar rats were randomly divided into four groups of five rats each. All the expressions of four genes were significantly higher in tooth than in mandibular bone and iliac bone.*

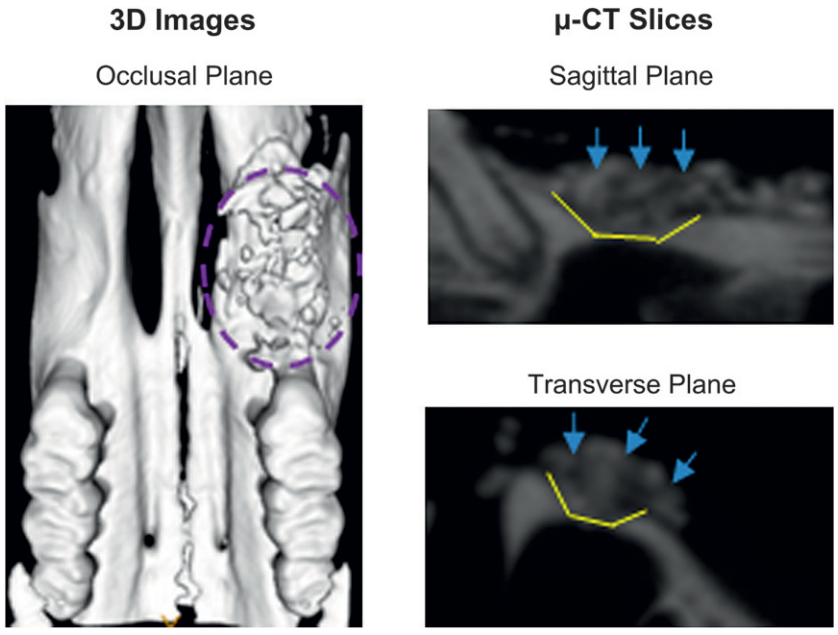

Figure 2.
μ-CT slices (80 kV/450 mA, 93-μm slice thickness) and three-dimensional images of the bone defect (2-mm diameter, 2-mm depth). Purple dashed line = part of the graft; yellow solid line = edge of the bone defect; blue arrowheads = graft material.

group, slight inflammatory reactions were similarly shown. The volume of new bone formation exceeded the defective bone region at 6 weeks. Furthermore, a large amount of bone marrow was formed compared to the tooth group. In the control group, little new bone was formed at either 6 or 8 weeks. No signs of inflammation were observed in any defects 8 weeks after surgery. New bone resorption at 8 weeks was marked compared to that at 6 weeks in the bone group. In contrast, new bone mostly filled the defects at 8 weeks in the tooth group (Fig. 4).

Immunohistochemical Observations

In the surroundings of the newly formed bone in both the tooth and bone groups at 6 and 8 weeks, OPN was more widely expressed. In the control group, there was little expression of OPN at both 6 and 8 weeks. DSP was expressed in the tooth fragment graft. In the bone defect, the expression was positive at 6 weeks, but hardly expressed at 8 weeks (Figs. 5 and 6).

DISCUSSION

We investigated the usefulness of teeth as a bone graft material for jawbone formation by comparing grafts done with teeth and autogenous iliac bone.

The treatment of defects made in the cranial bone of rats using graft materials has been reported.[18,30] However, it has been reported that some parts of the cranial bone are derived from developmentally different cells. Yoshida et al.[13] reported that the frontal bone is derived from neural crest cells, whereas the temporal bone is derived from the mesoderm. In accordance with Park et al.,[31] we used the jawbone for the graft bed.

We determined the evaluation times for μ-CT and histologic examination at 6 and 8 weeks after grafting based on

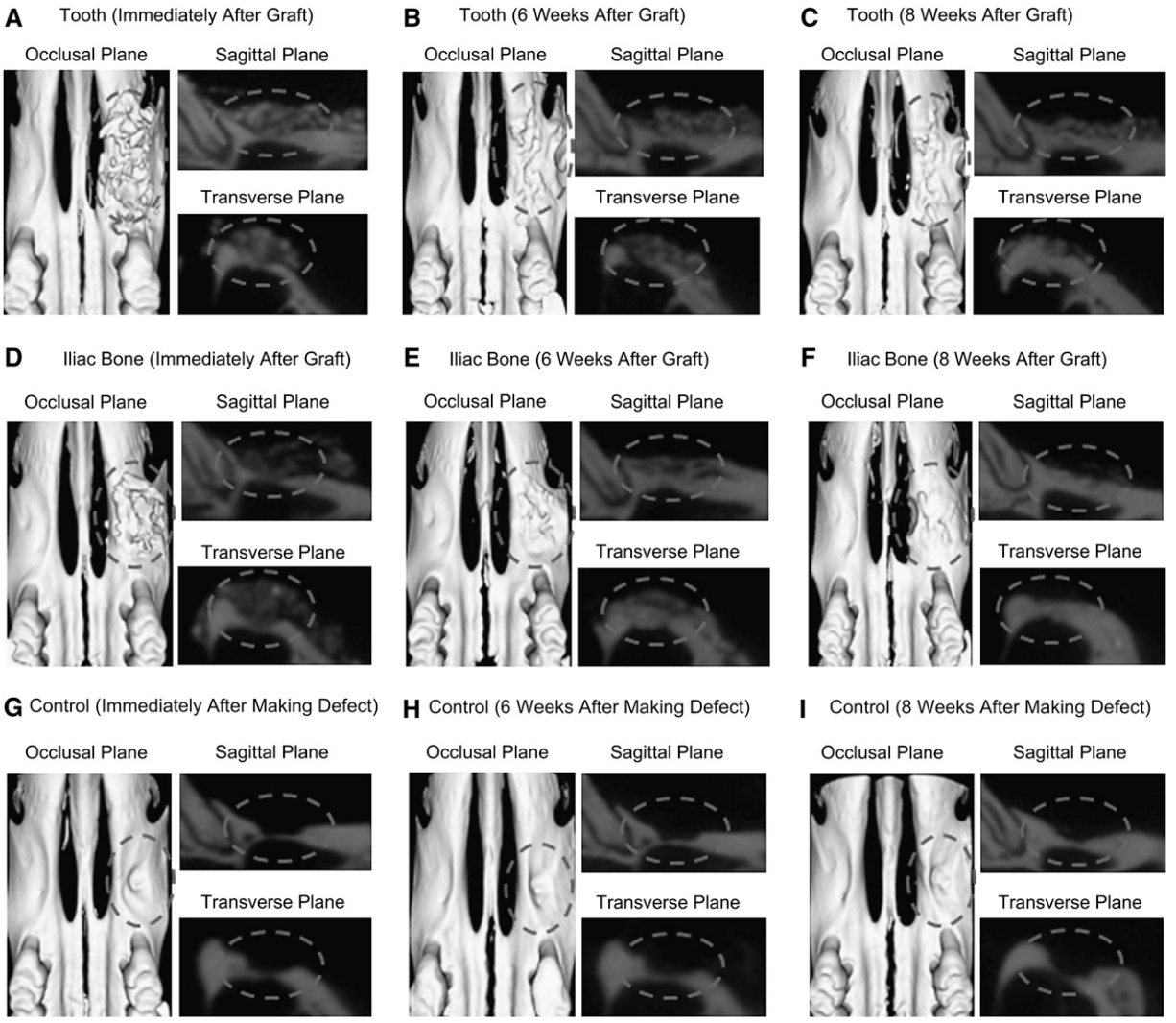

Figure 3.
*μ-CT images showing the tooth grafts immediately after surgery **(A)**, 6 weeks after surgery **(B)**, and 8 weeks after surgery **(C)**. μ-CT image showing the iliac bone grafts immediately after surgery **(D)**, 6 weeks after surgery **(E)**, and 8 weeks after surgery **(F)**. μ-CT image in control group (no graft) immediately after surgery **(G)**, 6 weeks after surgery **(H)**, and 8 weeks after surgery **(I)**.*

the healing process of rat jawbone tissue, as reported by Schmitz et al.[32] and Hyun et al.[33]

Shapoff et al.[34] reported that the particle size of graft materials influenced later bone formation. Bhaskar et al.[35] reported that the ideal particle size of bone graft materials is 500 μm and that the between-particle distance is 150 μm. These sizes were recommended because resorption requires a prolonged time if the particle size is too large, and particles are resorbed before they are able to function as a graft material if the size is too small. The retention of blood clots is difficult when the between-particle distance is too large, whereas blood vessels cannot readily enter the material when the distance is too small.[36] Because it was difficult to prepare particles with a homogeneous size from autogenous tissues, we added β-TCP to reduce the difference in the between-particle distance between the iliac bone grafts and extracted tooth grafts.

Enamel, an epithelial tissue, was completely removed from the teeth used for bone graft material. The remainder of the teeth including the dentin, cementum, pulp, and periodontal ligament were ground and immediately used for grafting.

Gene expressions of P75, P0, nestin, and musashi-1 were significantly higher in the tooth group than in mandibular and iliac bone groups using real-time PCR. P75 and P0 have been recently described as neural crest cell markers. P75 is the founding member of the tumor necrosis factor receptor superfamily. This family of receptors is distinguished by multiple cysteine-rich domains for ligand binding, a single transmembrane sequence, and a non-catalytic cytoplasmic

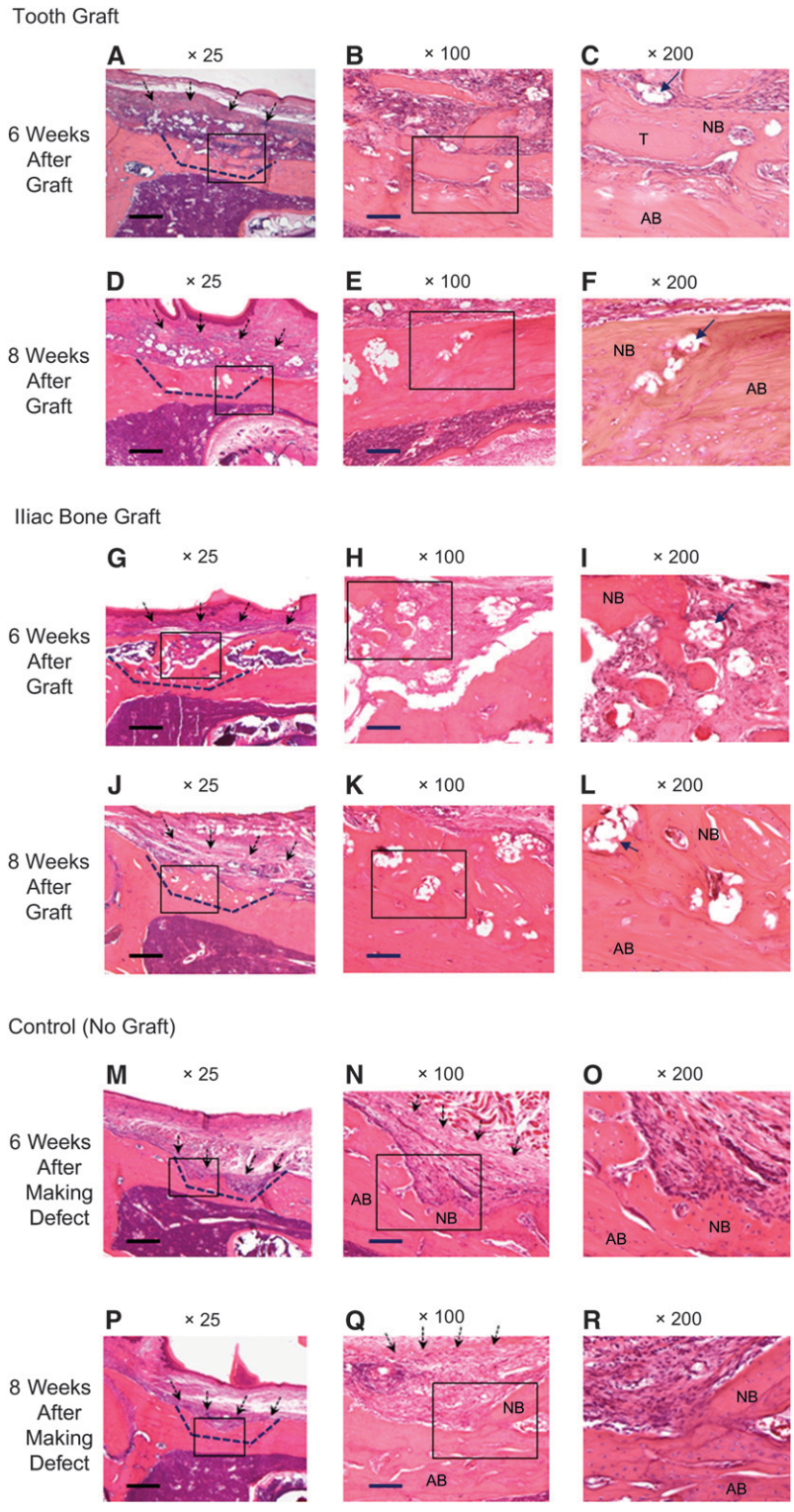

Figure 4.
Histology of bone regeneration 6 and 8 weeks after grafting. ***A through F)*** *Tooth graft (H&E, original magnification ×25, ×100, ×200, ×25, ×100, and ×200, respectively).* ***G through I)*** *Iliac bone graft (H&E, original magnification ×25, ×100, ×200, ×25, ×100, and ×200, respectively).* ***M through R)*** *Control (no graft) (H&E, original magnification ×25, ×100, ×200, ×25, ×100, and ×200, respectively). T = tooth; AB = alveolar bone; NB = new bone; dotted line = bone defect; blue arrowhead = β-TCP; black dotted arrowhead = collagen membrane; black scale bar = 100 μm; blue scale bar = 25 μm.*

domain. The P75 receptor is recognized by all the neurotrophins, which promote differentiation, growth, and survival of diverse cell types in the nervous system.[37] P0 is a cell-adhesion molecule of the immunoglobulin superfamily and is the main constituent of myelin sheaths in the peripheral nerve system.[38] Nestin and musashi-1 are marker proteins of central nervous stem cells. Nestin is an intermediate filament transiently expressed during neural ontogeny. In development, it is expressed first by neuroepithelial cells and radial glia, and later by progenitor cells of the ventricular zone (during the embryonic stage) and the nascent ependyma/subependyma (during the postnatal stage).[39] Musashi-1 is an RNA-binding protein, and its gene was formerly reported as another candidate marker gene for intestinal stem cells. Its molecular function has been determined as translational repression of target genes, such as *m-Numb*, a negative regulator of Notch signaling.[40] All genes were expressed at the highest levels in the tooth group followed by mandibular bone and iliac bone groups, but gene expressions were very low in the iliac bone, showing that the extracted tooth contained numerous undifferentiated neural crest–derived cells compared to the other tissues. Therefore, the extracted tooth graft seems to be more advantageous than grafts of the jawbone or the iliac bone.

Histology and μ-CT showed that new bone was formed and replaced with time (at 6 and 8 weeks) after extracted tooth grafting and that the dentin was incorporated into the new bone. In the iliac bone group, new bone was formed, and marked formation of the bone marrow structure in the new bone was noted. We prepared specimens from intact rats and observed the alveolar bone structure in the region corresponding to the bone-defective region. No bone marrow structure was present in the intact rat upper alveolar bone. Akintoye et al.[41] reported that the properties of bone marrow stromal cells of the iliac bone are different from those of the maxilla and mandible, and bone marrow structure formation was marked when the iliac bone was grafted compared to that after jawbone grafting, because the iliac bone contains more red marrow.

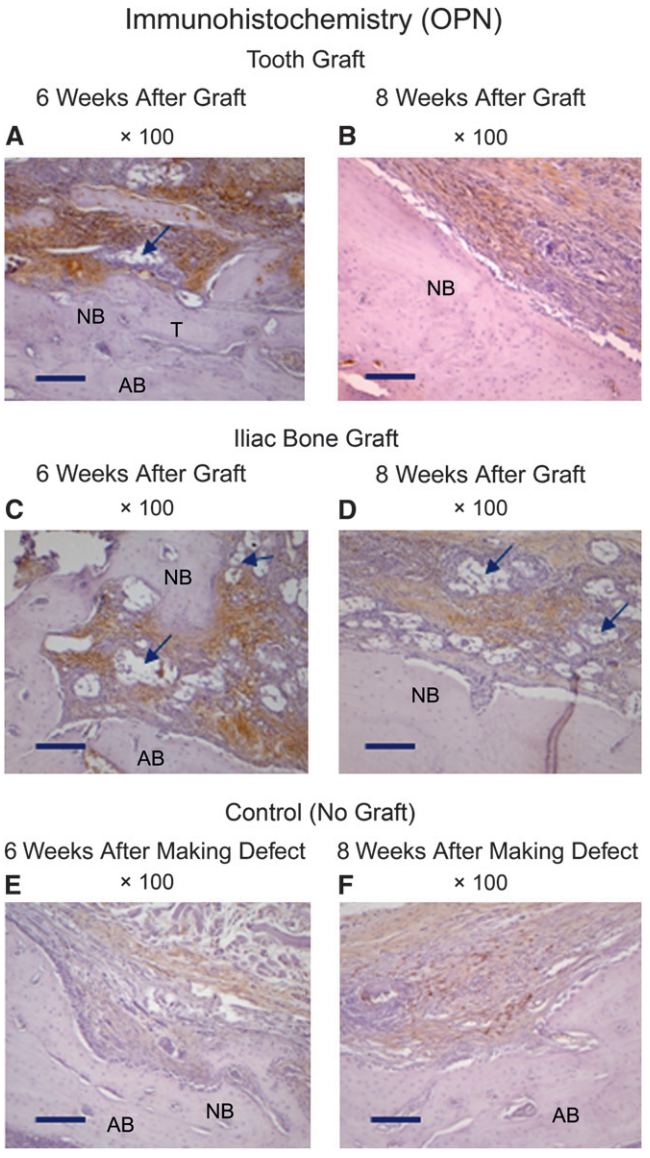

Figure 5.
*Comparison of immunohistochemical observation for OPN. **A** and **B)** Tooth graft; **C** and **D)** Iliac bone graft; **E** and **F)** Control (no graft). T = tooth; AB = alveolar bone; NB = new bone; arrowheads = β-TCP; blue scale bar = 25 μm (H&E, original magnification × 100).*

Moreover, on μ-CT and histologic investigation, new bone and bone marrow structure formation was marked 6 weeks after iliac bone grafting, but the bone marrow structure had contracted at 8 weeks. Furthermore, resorption of newly formed bone was greater at 8 weeks than at 6 weeks. Resorption of new formed bone after iliac bone grafting was marked compared to tooth grafting, showing that the iliac bone induced new bone earlier. However, a large amount of the new bone was subsequently resorbed, resulting in similar new bone mass to that formed by tooth grafting at 8 weeks. This was consistent with that reported by Donos et al.[10] after iliac bone grafting. A typical rat from each treatment group is shown in Figures 1 through 6. All rats within each experimental group exhibited similar trends by histology and μ-CT analyses. We used a membrane because it is often used in cases of clinical bone repair. However, the use of a foreign body membrane may not be fully advantageous for wound healing, because it compromises the periosteum, an important provider of osteogenic cells in the bone formation process.

OPN promotes the early differentiation of osteoblasts, their adhesion to bone, and bone formation. It also promotes bone resorption by promoting the adhesion of osteoclasts to the bone surface.[42,43] On immunohistochemical staining with anti-OPN antibody, OPN was clearly expressed in both iliac bone and tooth grafts at 6 and 8 weeks, suggesting active new bone formation. In the control group, only a few expressions were detected at 6 and 8 weeks. We believe that the hard tissue formed within the defective area in the tooth group were not tooth fragments but the osseous tissue formed with remodeling of bone.

DSP is a dentin-specific non-collagenous protein involved in the calcification of dentin. It is similar to sialoproteins, such as OPN, BSP, and DMP-1, and its presence in bone has been shown, although at a very low level.[44,45] The precursor protein of DSP, dentin sialophosphoprotein, is known to be involved with bone calcification.[46] On immunohistochemical staining with anti-DSP antibody, the positive reaction was localized to the dentin of the extracted tooth fragments incorporated into the new bone at 6 weeks, suggesting that dentin has a high affinity for and marked osteoconductive effect on jawbone. The DSP-positive area narrowed at 8 weeks. It was clarified that mechanisms of resorption for biodegradable and osteoinductive materials include the initial resorption of the material after transplantation and the subsequent ingrowth of new bone into the resorbed regions. In terms of DSP-positive areas, the possibility for granules of teeth to be resorbed with the time passage and to be substituted with osseous tissue was also clarified because the DSP-positive areas decreased from 6 weeks to 8 weeks.

Teeth contain dentin, dental pulp, cementum, and the periodontal ligament. Ike and Urist[47] performed a bone regeneration experiment using recombinant human BMP-2, in which the use of decalcified dried dentin for scaffolding resulted in new bone formation. Dentin contains growth factors, such as IGF-II, BMP-2, and TGF-β, similar to bone.[20] Saygin et al.[21] reported that in cementum, cementoblasts contain TGF-β, IGF-I, and PDGF-BB. The periodontal ligament also contains TGF-β, IGF-I, basic fibroblast growth factor, vascular endothelial growth factor, BMP-2, PDGF, and type I collagen.[24]

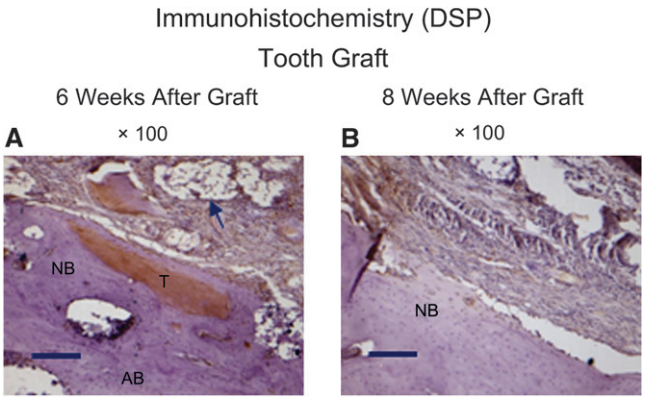

Figure 6.
Immunohistochemical observation for DSP. ***A)*** *At 6 weeks, new bone induction occurred around pieces of the tooth.* ***B)*** *At 8 weeks, DSP staining was immunonegative. T = tooth; AB alveolar bone; NB = new bone; arrowhead = β-TCP; bar = 25 μm (H&E, original magnification × 100).*

Many proteins are common to bone, dentin, and cementum. In addition to OPN and DSP, BSP, osteocalcin, DMP-1, type I collagen, osterix, and Runx2 are common, and these proteins are reportedly involved in bone formation and resorption.[48] Therefore, many constituents of teeth are proteins or growth factors involved in bone formation.

In a clinical setting, teeth from areas in which there is potential infection cannot be used. In particular, it is necessary to avoid the use of teeth with an infected root canal, root side caries, or an inflammation and a cyst in the surrounding periodontal tissue. It is thought that it is necessary to remove the enamel, caries, or the part with the infectious risk completely before a tooth is extracted for this purpose.

CONCLUSION

The results of this study suggest that material made from extracted teeth may have potential as a bone graft material for jawbone formation, because it is highly predictable and shows less resorption after grafting.

ACKNOWLEDGMENTS

The authors thank Dr. Tetsuo Suzawa (Department of Oral Biochemistry, School of Dentistry, Showa University, Tokyo, Japan). This study was supported by the High-Tech Research Center Project for Private Universities and in part by the Special Subsidies in Subsidies for ordinary expenses of private schools from the Promotion and Mutual Aid Corporation for Private Schools of Japan. Equipment was provided by the Animal Research Committee of Showa University. The authors report no conflicts of interest related to this study.

REFERENCES

1. Nasr HF, Aichelmann-Reidy ME, Yukna RA. Bone and bone substitutes. *Periodontol 2000* 1999;19:74-86.
2. Quattlebaum JB, Mellonig JT, Hensel NF. Antigenicity of freeze-dried cortical bone allograft in human periodontal osseous defects. *J Periodontol* 1988;59:394-397.
3. Kamijou T, Nakajima T, Ozawa H. Effects of osteocytes on osteoinduction in the autogenous rib graft in the rat mandible. *Bone* 1994;15:629-637.
4. Schallhorn RG. Long term evaluation of osseous grafts in periodontal therapy. *Int Dent J* 1980;30:101-116.
5. Schallhorn RG. Eradication of bifurcation defects utilizing frozen autogenous hip marrow implants. *Periodontal Abstr* 1967;15:101-105.
6. Zins JE, Whitaker LA. Membranous versus endochondral bone: Implications for craniofacial reconstruction. *Plast Reconstr Surg* 1983;72:778-785.
7. Borstlap WA, Heidbuchel KL, Freihofer HP, Kuijpers-Jagtman AM. Early secondary bone grafting of alveolar cleft defects. A comparison between chin and rib grafts. *J Craniomaxillofac Surg* 1990;18:201-205.
8. Koole R, Bosker H, van der Dussen FN. Late secondary autogenous bone grafting in cleft patients comparing mandibular (ectomesenchymal) and iliac crest (mesenchymal) grafts. *J Craniomaxillofac Surg* 1989; 17 (Suppl. 1):28-30.
9. Donovan MG, Dickerson NC, Hellstein JW, Hanson LJ. Autologous calvarial and iliac onlay bone grafts in miniature swine. *J Oral Maxillofac Surg* 1993;51:898-903.
10. Donos N, Kostopoulos L, Tonetti M, Karring T. Long-term stability of autogenous bone grafts following combined application with guided bone regeneration. *Clin Oral Implants Res* 2005;16:133-139.
11. Chung IH, Yamaza T, Zhao H, Choung PH, Shi S, Chai Y. Stem cell property of postmigratory cranial neural crest cells and their utility in alveolar bone regeneration and tooth development. *Stem Cells* 2009;27:866-877.
12. Nagoshi N, Shibata S, Kubota Y, et al. Ontogeny and multipotency of neural crest-derived stem cells in mouse bone marrow, dorsal root ganglia, and whisker pad. *Cell Stem Cell* 2008;2:392-403.
13. Yoshida T, Vivatbutsiri P, Morriss-Kay G, Saga Y, Iseki S. Cell lineage in mammalian craniofacial mesenchyme. *Mech Dev* 2008;125:797-808.
14. Morrison SJ, White PM, Zock C, Anderson DJ. Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. *Cell* 1999;96:737-749.
15. Stevens A, Zuliani T, Olejnik C, et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. *Stem Cells Dev* 2008;17:1175-1184.
16. Miletich I, Sharpe PT. Neural crest contribution to mammalian tooth formation. *Birth Defects Res C Embryo Today* 2004;72:200-212.
17. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. *Stem Cells* 2008;26:1787-1795.
18. Seo BM, Sonoyama W, Yamaza T, et al. SHED repair critical-size calvarial defects in mice. *Oral Dis* 2008; 14:428-434. (erratum 2009;15:302).
19. Schmidt-Schultz TH, Schultz M. Intact growth factors are conserved in the extracellular matrix of ancient

human bone and teeth: A storehouse for the study of human evolution in health and disease. *Biol Chem* 2005;386:767-776.
20. Gao J, Symons AL, Bartold PM. Expression of transforming growth factor-beta 1 (TGF-beta1) in the developing periodontium of rats. *J Dent Res* 1998;77: 1708-1716.
21. Saygin NE, Tokiyasu Y, Giannobile WV, Somerman MJ. Growth factors regulate expression of mineral associated genes in cementoblasts. *J Periodontol* 2000;71:1591-1600.
22. Isaka J, Ohazama A, Kobayashi M, et al. Participation of periodontal ligament cells with regeneration of alveolar bone. *J Periodontol* 2001;72:314-323.
23. Flores MG, Yashiro R, Washio K, Yamato M, Okano T, Ishikawa I. Periodontal ligament cell sheet promotes periodontal regeneration in athymic rats. *J Clin Periodontol* 2008;35:1066-1072.
24. Emecen P, Akman AC, Hakki SS, et al. ABM/P-15 modulates proliferation and mRNA synthesis of growth factors of periodontal ligament cells. *Acta Odontol Scand* 2009;67:65-73.
25. Hoeppner LH, Secreto F, Jensen ED, Li X, Kahler RA, Westendorf JJ. Runx2 and bone morphogenic protein 2 regulate the expression of an alternative Lef1 transcript during osteoblast maturation. *J Cell Physiol* 2009;221:480-489.
26. Handschin AE, Egermann M, Trentz O, et al. Cbfa-1 (Runx-2) and osteocalcin expression by human osteoblasts in heparin osteoporosis in vitro. *Clin Appl Thromb Hemost* 2006;12:465-472.
27. Tsukamoto-Tanaka H, Ikegame M, Takagi R, Harada H, Ohshima H. Histochemical and immunocytochemical study of hard tissue formation in dental pulp during the healing process in rat molars after tooth replantation. *Cell Tissue Res* 2006;325:219-229.
28. Takamori Y, Suzuki H, Nakakura-Ohshima K, et al. Capacity of dental pulp differentiation in mouse molars as demonstrated by allogenic tooth transplantation. *J Histochem Cytochem* 2008;56:1075-1086.
29. Hasegawa T, Suzuki H, Yoshie H, Ohshima H. Influence of extended operation time and of occlusal force on determination of pulpal healing pattern in replanted mouse molars. *Cell Tissue Res* 2007;329:259-272.
30. Intini G, Andreana S, Buhite RJ, Bobek LA. A comparative analysis of bone formation induced by human demineralized freeze-dried bone and enamel matrix derivative in rat calvaria critical-size bone defects. *J Periodontol* 2008;79:1217-1224.
31. Park CH, Abramson ZR, Taba M Jr, et al. Three-dimensional micro-computed tomographic imaging of alveolar bone in experimental bone loss or repair. *J Periodontol* 2007;78:273-281.
32. Schmitz JP, Schwartz Z, Hollinger JO, Boyan BD. Characterization of rat calvarial nonunion defects. *Acta Anat (Basel)* 1990;138:185-192.
33. Hyun SJ, Han DK, Choi SH, et al. Effect of recombinant human bone morphogenetic protein-2, -4, and -7 on bone formation in rat calvarial defects. *J Periodontol* 2005;76:1667-1674.
34. Shapoff CA, Bowers GM, Levy B, Mellonig JT, Yukna RA. The effect of particle size on the osteogenic activity of composite grafts of allogeneic freeze-dried bone and autogenous marrow. *J Periodontol* 1980;51: 625-630.
35. Bhaskar SN, Cutright DE, Knapp MJ, Beasley JD, Perez B, Driskell TD. Tissue reaction to intrabony ceramic implants. *Oral Surg Oral Med Oral Pathol* 1971;31:282-289.
36. Topazian RG, Hammer WB, Boucher LJ, Hulbert SF. Use of alloplastics for ridge augmentation. *J Oral Surg* 1971;29:792-798.
37. Parkhurst CN, Zampieri N, Chao MV. Nuclear localization of the p75 neurotrophin receptor intracellular domain. *J Biol Chem* 2010;285:5361-5368.
38. Iwao K, Inatani M, Okinami S, Tanihara H. Fate mapping of neural crest cells during eye development using a protein 0 promoter-driven transgenic technique. *Graefes Arch Clin Exp Ophthalmol* 2008;246: 1117-1122.
39. Kawaguchi A, Miyata T, Sawamoto K, et al. Nestin-EGFP transgenic mice: Visualization of the self-renewal and multipotency of CNS stem cells. *Mol Cell Neurosci* 2001;17:259-273.
40. Murayama M, Okamoto R, Tsuchiya K, et al. Musashi-1 suppresses expression of Paneth cell-specific genes in human intestinal epithelial cells. *J Gastroenterol* 2009; 44:173-182.
41. Akintoye SO, Lam T, Shi S, Brahim J, Collins MT, Robey PG. Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. *Bone* 2006;38:758-768.
42. Ono N, Nakashima K, Rittling SR, et al. Osteopontin negatively regulates parathyroid hormone receptor signaling in osteoblasts. *J Biol Chem* 2008;283: 19400-19409.
43. Ihara H, Denhardt DT, Furuya K, et al. Parathyroid hormone-induced bone resorption does not occur in the absence of osteopontin. *J Biol Chem* 2001;276: 13065-13071.
44. Suzuki S, Sreenath T, Haruyama N, et al. Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biol* 2009;28: 221-229.
45. Qin C, Brunn JC, Cadena E, et al. The expression of dentin sialophosphoprotein gene in bone. *J Dent Res* 2002;81:392-394.
46. Verdelis K, Ling Y, Sreenath T, et al. DSPP effects on in vivo bone mineralization. *Bone* 2008;43:983-990.
47. Ike M, Urist MR. Recycled dentin root matrix for a carrier of recombinant human bone morphogenetic protein. *J Oral Implantol* 1998;24:124-132.
48. Ye L, MacDougall M, Zhang S, et al. Deletion of dentin matrix protein-1 leads to a partial failure of maturation of predentin into dentin, hypomineralization, and expanded cavities of pulp and root canal during postnatal tooth development. *J Biol Chem* 2004;279: 19141-19148.

Correspondence: Dr. Junichi Watahiki, Department of Orthodontics, School of Dentistry, Showa University, 2-1-1 Kitasenzoku, Ohta-ku, Tokyo, 145-8515, Japan. Fax: 81-03-3784-6641; e-mail: junwata2000@ybb.ne.jp.

Submitted January 13, 2010; accepted for publication March 26, 2010.