ORAL DISEASES

Oral Diseases (2011) 17, 379–386 doi:10.1111/j.1601-0825.2010.01761.x

www.wiley.com

ORIGINAL ARTICLE

Root and periodontal tissue development after allogenic tooth transplantation between rat littermates

K Andou[1,2], M Nakamura[2], Y Ina[1], K Sasaki[1], Y Sasano[2]

[1]*Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry, Sendai;* [2]*Divison of Craniofacial Development and Regeneration, Tohoku University Graduate School of Dentistry, Sendai, Japan*

OBJECTIVE: The study was designed to investigate the development of roots and periodontal tissues after allogenic tooth transplantation between rat littermates by micro-computed tomography (micro-CT) and histology.

MATERIALS AND METHODS: The upper right second molars in 2-week-old rats were extracted and immediately transplanted into the upper right first molar socket of rat littermates under anesthesia. The upper left second molars in 2-week-old recipient rats were used as a control. The rats were fixed and tissues analyzed at 0, 4, 8, or 12 weeks after transplantation. Root development of seven rats in each group was analyzed quantitatively using micro-CT. Periodontal tissue formation was examined qualitatively by histologic methods.

RESULTS: Roots developed after allogenic transplantation, but they were significantly shorter than control roots. The number of roots varied from one to four in transplanted teeth, while it was consistently four in control teeth. Periodontal tissue formation in transplanted teeth was equivalent to that of the control teeth.

CONCLUSION: Allogenic transplantation between rat littermates permits root development and periodontal tissue formation.

Oral Diseases (2011) **17**, 379–386

Keywords: tooth; micro-CT; histology; root development; periodontal tissue formation; allogenic transplantation; rats

Introduction

Clinical data based on X-ray analysis show survival and continuous root development of teeth transplanted autogenously (Myrlund *et al*, 2004; Bauss *et al*, 2005). In contrast, allogenically transplanted teeth have been reported to cause irreversible root resorption, followed by the loss of the tooth graft (Nordenram, 1982; Schwartz *et al*, 1987). Mouse teeth transplanted into tooth sockets allogenically have shown inflammatory pulp tissue and poor arrangement of periodontal ligaments, and the process often results in immune rejection (Kim *et al*, 2006; Unno *et al*, 2009).

Little information is available about development of roots and periodontal tissues in teeth transplanted allogenically in the experimental model. We do not know by how much roots elongate, how many roots are developed in transplanted teeth, or whether transplanted teeth develop physiologically normal roots and periodontal tissue. We wanted to know whether roots and periodontal tissue develop physiologically when a tooth is transplanted allogenically into a recipient provided that the immune response is regulated. Reports have shown low rejection rates of transplanted teeth after experimental allogenic transplantation between mouse littermates (Unno *et al*. 2009), which may provide a useful experimental model for analyzing root and periodontal tissue development in allogenic transplantation.

Micro-computed tomography (micro-CT) is a rapid, reproducible, and non-invasive method for observation of structures in bones and teeth, giving results that are closely comparable with those obtained by histology. Micro-CT has potential for three-dimensional reconstruction of detailed tooth structure and has been used to analyze sophisticated morphology of the teeth in experimental animals such as mice and rats (Balto *et al*, 2002; Shibata *et al*, 2004; Tsukamoto-Tanaka *et al*, 2006; Ebina *et al*, 2009).

The present study was designed to investigate root development quantitatively using micro-CT and periodontal tissue formation qualitatively with histology in allogenic tooth transplantation between rat littermates.

Materials and methods

Animals and transplantation

The guidelines for animal users (NIH Animal Research Advisory Committee, 1995) and specific national laws

Correspondence: Yasuyuki Sasano, DDS, PhD, Division of Craniofacial Development and Regeneration, Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan. Tel: +81 22 717 8285, Fax: +81 22 717 8288, E-mail: sasano@anat.dent.tohoku.ac.jp
Received 10 May 2010; revised 20 July 2010; accepted 3 August 2010

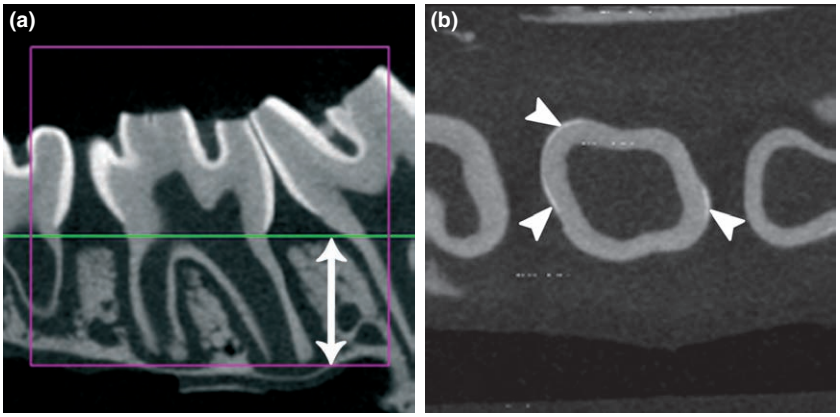

Figure 1 The distance from the boundary between a crown and roots to the apex of the longest root is defined as root length (**a**, up–down arrow). The transverse plane of computed tomography (CT) sliced views is tilted to reveal enamel evenly around the crown dentin and is moved toward roots until the last enamel (**b**, arrowheads) is unrecognizable, which is defined as the boundary between the crown and roots

were followed. Preparation was according to institutional animal protocols approved by Tohoku University. Wistar rat littermates aged 2 weeks (which was designated as week 0) were used. Female rats were used as donors and male rats as recipients.

Recipient rats were anesthetized with sodium pentobarbital (12.5 mg kg^{-1}) intraperitoneally with supplemental ether inhalation. Donor rats were killed under deep anesthesia, and an upper right second molar was extracted and immediately transplanted into the upper right first molar socket of the recipient rat after the first molar had been extracted. The upper left second molar of the recipient rat served as a control. All the experiments were carried out under aseptic conditions. The rats were maintained on a soft diet (Ina *et al*, 2008).

Tissue preparation

The recipient rats were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer by perfusion through the aorta under anesthesia at 4, 8 and 12 weeks after transplantation. The maxillae and subcutaneous tissues, including transplanted and control teeth, were resected and kept in the same fixative overnight at 4°C. The specimens were then kept in 0.1 M phosphate buffer at 4°C before examination by micro-CT. Non-recipient rats aged 2 weeks postnatally were processed in the same way to examine upper second molars at week 0 after transplantation.

The specimens examined by micro-CT were then decalcified and embedded in paraffin, serial sections were cut, and adjacent sections were stained with hematoxylin–eosin (H&E).

Micro-CT examination

The specimens were analyzed by micro-CT (Scan Xmate-E090; Comscan, Kanagawa, Japan) under standardized conditions. The CT settings were as follows: pixel matrix, $516 \times 516 \times 506$; slice thickness, 14.281 μm; magnification, $\times 7.0$; voltage, 90 kV; electrical current, 112 μA; and electrical power, 10 W. The samples were reconstructed using a software program (Tri3DBon64; Ratoc, Tokyo, Japan) to evaluate the tooth root length and the number of roots of control and transplanted teeth.

The transverse plane of CT sliced views was tilted to reveal enamel evenly around crown dentin and moved toward the roots until the last enamel was unrecognizable, which was defined as a three-dimensional boundary between a crown and roots. The distance between the boundary and the apex of the longest root was defined as the root length (Figure 1). Reconstructed CT images were used to analyze the morphology of the teeth and the number of roots in control and transplanted teeth.

Statistical analysis

Seven specimens for each condition were analyzed statistically using SPSS 16.0J for Windows (SPSS Japan Inc, Tokyo, Japan). The Kruskal–Wallis test was used to compare the control and experimental groups. The level of statistical significance was taken as $P < 0.05$. Then the differences within the control and experimental samples were analyzed by multiple comparisons using Mann–Whitney *U*-test and statistical significance was set after Bonferroni correction ($P < 0.008$). Corresponding samples in the control and experimental groups were compared using Mann–Whitney *U*-test with $P < 0.05$.

Results

The data were obtained from seven teeth for each of the control and transplanted groups at each week (0, 4, 8, and 12 weeks) after transplantation.

Morphology of transplanted teeth obtained from micro-CT analysis

Formation of the crown was completed and elongation of the root began at week 0, when the root did not show any branching (Figure 2). Four roots, mesiobuccal (mb), mesiolingual (ml), distobuccal (db), and distolingual (dl) roots, were formed by week 4 in control teeth. In contrast, the number of roots varied from one to four in transplanted teeth (Figures 2 and 3), of which the average was 2.1, 2.9, and 3.1 at weeks 4, 8, and 12 respectively (Table 1). We identified which root of the transplanted tooth corresponds to each of four roots of the control tooth when the transplanted tooth developed four roots (Figure 2). Only one tooth developed four

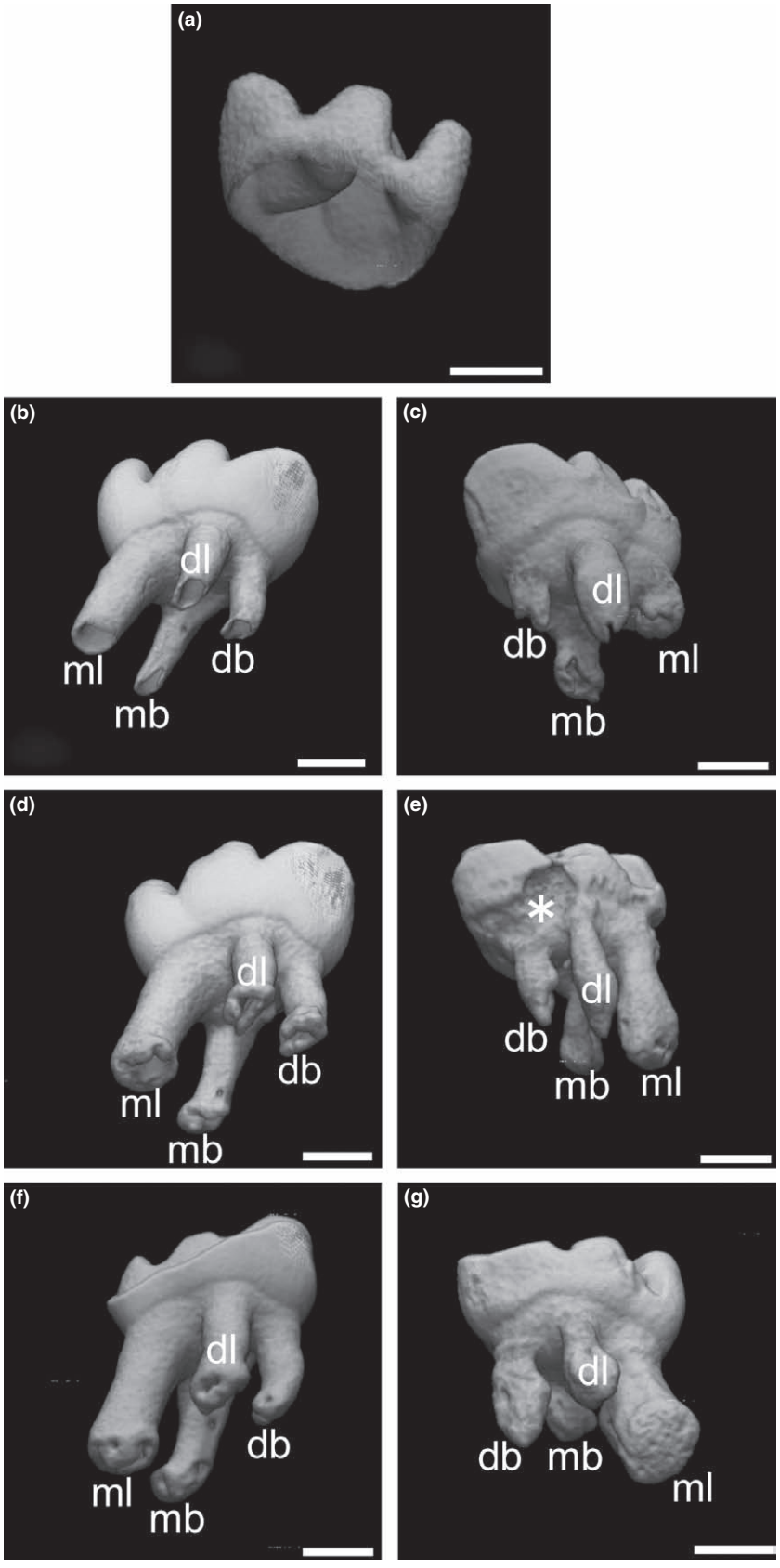

Figure 2 Images of control and transplanted teeth obtained by micro-computed tomography (micro-CT). Formation of the crown is completed at week 0, when the root does not show any branching (**a**). Control teeth develop four roots, mesiobuccal (mb), mesiolingual (ml), distobuccal (db), and distolingual (dl) roots, at weeks 4 (**b**), 8 (**d**), and 12 (**f**). Transplanted teeth with four roots at weeks 4 (**c**), 8 (**e**), and 12 (**g**) are also shown. The surfaces of roots and crowns of transplanted teeth are coarse and are often with some defects (asterisk). Scale bars = 1000 μm

Table 1 The average number of roots per tooth. $n = 7$ for each condition – weeks 4, 8, and 12 – in control and transplanted teeth

	Average ± *s.d.*
Control tooth (weeks 4, 8, 12)	4 ± 0
Transplanted tooth (week 4)	2.1 ± 1.2
Transplanted tooth (week 8)	2.9 ± 1.1
Transplanted tooth (week 12)	3.1 ± 0.9

roots out of seven transplanted teeth at week 4 and only three transplanted teeth out of seven developed four roots at weeks 8 and 12, respectively. In contrast, when transplanted teeth developed only three, two, or one root(s), we were unable to identify each of them as seen in Figure 3. As the number of roots that were unable to be identified was so large in the transplanted teeth, we analyzed only the longest root as described below.

The morphology of the transplanted teeth was not as uniform as that of the control teeth, and the surfaces of roots and crown were coarse, with some defects (Figure 2).

In total, 31 teeth were transplanted, of which four were not maintained in the maxilla when examined by micro-CT, another four extensively absorbed, and two adhered to the bone. Those specimens were excluded from micro-CT analysis.

Quantitative analysis of root length

The roots at week 4 were significantly longer than at week 0 and shorter than at week 8 in control teeth, whereas the length showed no difference between weeks 8 and 12. The roots of transplanted teeth were significantly longer at week 4 than at week 0 but showed no difference among weeks 4, 8, and 12. The roots at weeks 4, 8, and 12 in transplanted teeth were significantly shorter than those of corresponding controls (Figure 4).

Histology

Histologic images corresponded to those obtained by micro-CT in both control (Figure 5) and transplanted (Figure 6) teeth. Crown formation was almost complete at week 0 in control teeth, when enamel matrix was recognized in sections of decalcified specimens. Erupted teeth with elongated roots were observed in week 4 controls, and the enamel matrix was no longer identified. Cementum on the surface of roots, with surrounding periodontal ligaments and alveolar bone, was observed in controls at weeks 4, 8, and 12 (Figure 5).

Transplanted teeth had erupted and roots elongated by week 4, and the enamel matrix remained in the crown (Figure 6b). Crown dentin was thicker at weeks 8 (Figure 6e) and 12 (Figure 6i), whereas the enamel matrix still remained. The dentin matrix in the transplanted teeth was divided into two layers by a line deeply stained with hematoxylin. The line indicated the boundary between dentin formed before transplantation and dentin formed after transplantation (Akiba *et al*, 2006). Cementum and surrounding periodontal ligaments and alveolar bone were observed.

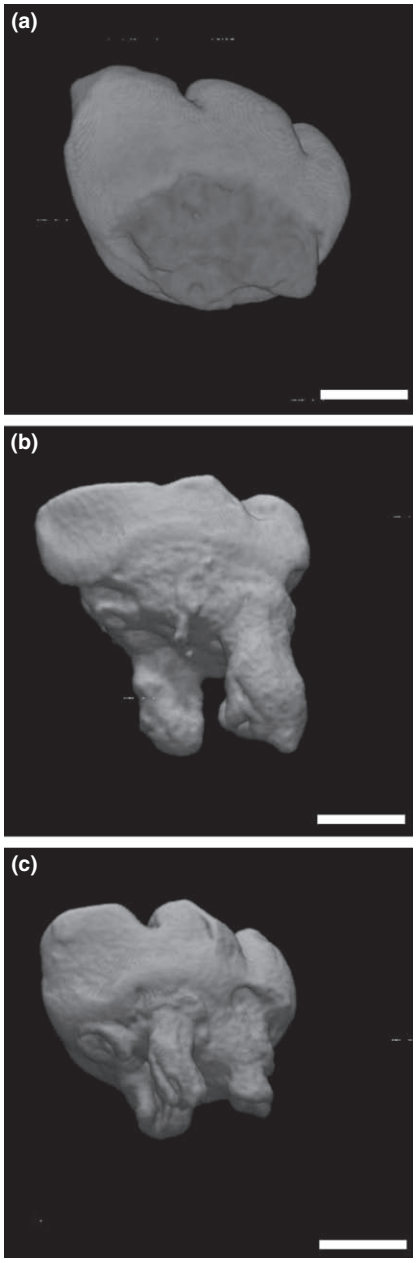

Figure 3 Images of transplanted teeth obtained by micro-CT. The figures show examples of 1 (**a**), 2 (**b**), and 3 (**c**) roots, at week 4 (**a**), week 8 (**b**), and week 4 (**c**). Scale bars = 1000 μm

The transplanted teeth (21 teeth) analyzed by micro-CT were examined in serial sections histologically. About 40% showed degeneration of dental pulp tissue (Figure 7a), about a half of which were accompanied by inflammatory periodontal ligaments (Figure 7b). The other specimens showed sound tissues on histologic examination.

Discussion

Previous studies have utilized micro-CT for sophisticated analysis of the morphology of teeth in experimental animals such as mice and rats (Balto *et al*, 2002;

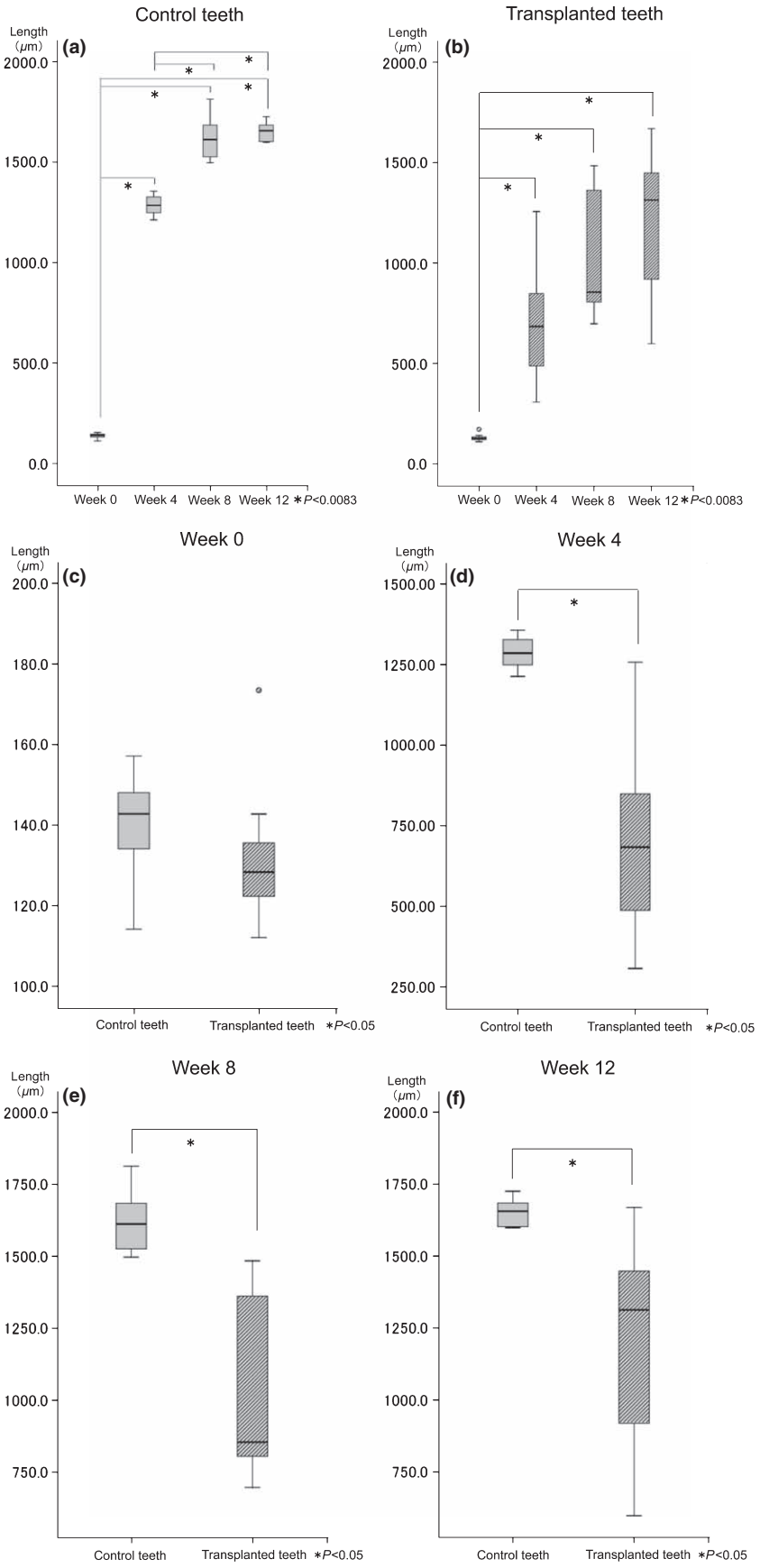

Figure 4 Quantitative analysis of the root length. In control teeth (**a**), the roots at week 4 are significantly longer than that at week 0 and smaller than at week 8, whereas the length shows no difference between weeks 8 and 12. In transplanted teeth (**b**), the roots are significantly longer at week 4 than at week 0 but show no difference among weeks 4, 8, and 12. The roots at weeks 4 (**d**), 8 (**e**), and 12 (**f**) in transplanted teeth are significantly shorter than those of corresponding controls but the length of roots at week 0 shows no significant difference (**c**)

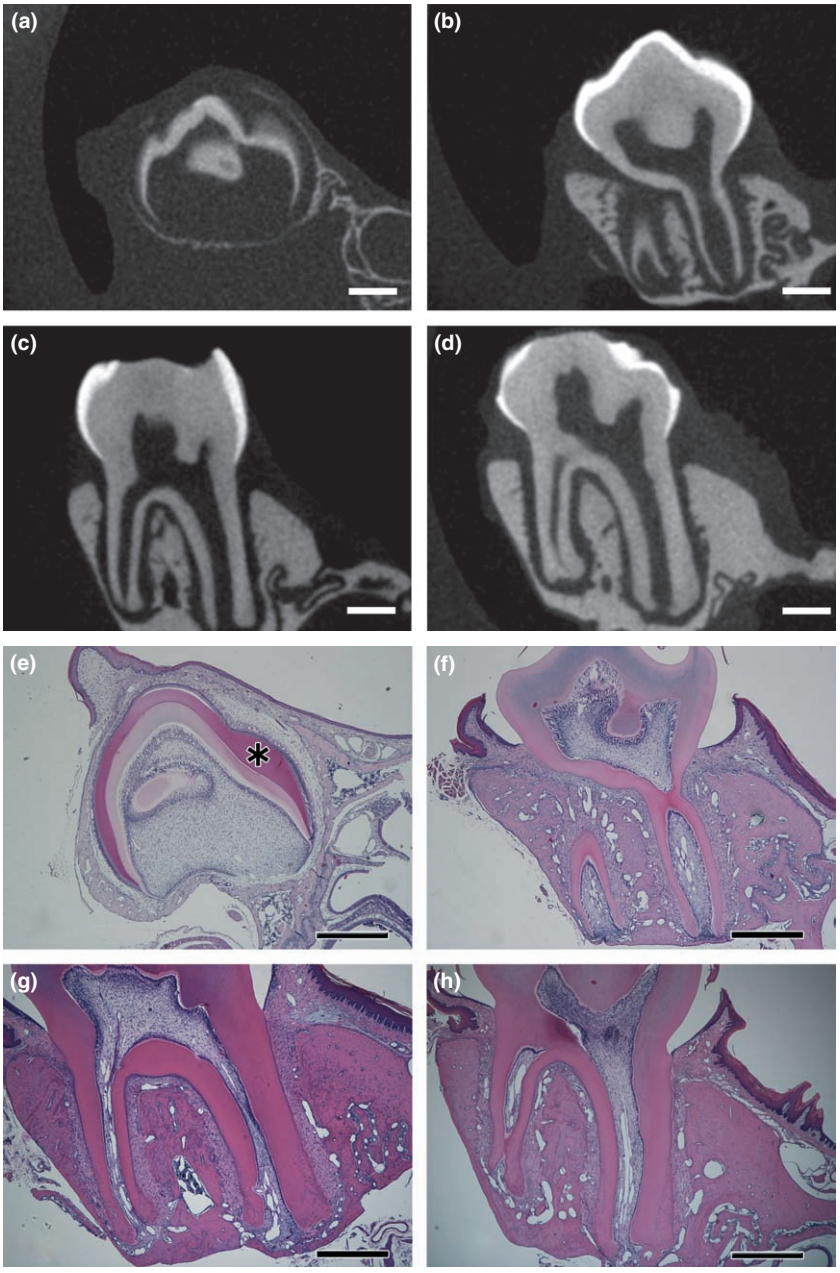

Figure 5 Micro-computed tomography (micro-CT) images (**a**–**d**) and histology (**e**–**h**) of control teeth at weeks 0 (**a**, **e**), 4 (**b**, **f**), 8 (**c**, **g**), and 12 (**d**, **h**). Histologic images correspond to those obtained by micro-CT. Crown formation is almost complete at week 0, when enamel matrix is recognized in decalcified sections (asterisk). Erupted teeth with elongated roots are observed at week 4, and the enamel matrix is no longer identified. Cementum on the surface of roots, with surrounding periodontal ligaments and alveolar bone, is observed at weeks 4, 8, and 12. Scale bars = 500 μm

Shibata *et al*, 2004; Tsukamoto-Tanaka *et al*, 2006; Ebina *et al*, 2009). We examined the number and quantified the length of roots in experimentally transplanted teeth using micro-CT and found that the roots and periodontal tissues developed, although roots were shorter and fewer than in the control teeth.

Our previous study, in which an unerupted lower second molar was transplanted autogenously into the upper right first molar socket in male Wistar rats at 2 weeks postnatally, showed formation of roots and periodontal ligaments (Akiba *et al*, 2006). Information is not available about formation of roots and periodontal tissues in experimentally allogenic tooth transplantation. The present study used transplantation between littermates to reduce the likelihood of immune rejection (Unno *et al*, 2009) and demonstrated experimentally for the first time that allogenic transplantation allows root development and periodontal tissue formation.

Our previous study showed that micro-CT was a suitable tool for quantitative analysis of the three-dimensional structure of small teeth of rats (Ebina *et al*, 2009). The present study examined the length and number of roots of experimentally transplanted teeth using micro-CT. In this study, micro-CT showed that allogenic transplantation could be followed by root development. The roots were significantly shorter and the number was smaller than those of corresponding controls. Moreover, the morphology of transplanted teeth was not as uniform as that of controls, and the surfaces of roots and crown were coarse, with some defects. Odontoclasts may be involved in the formation

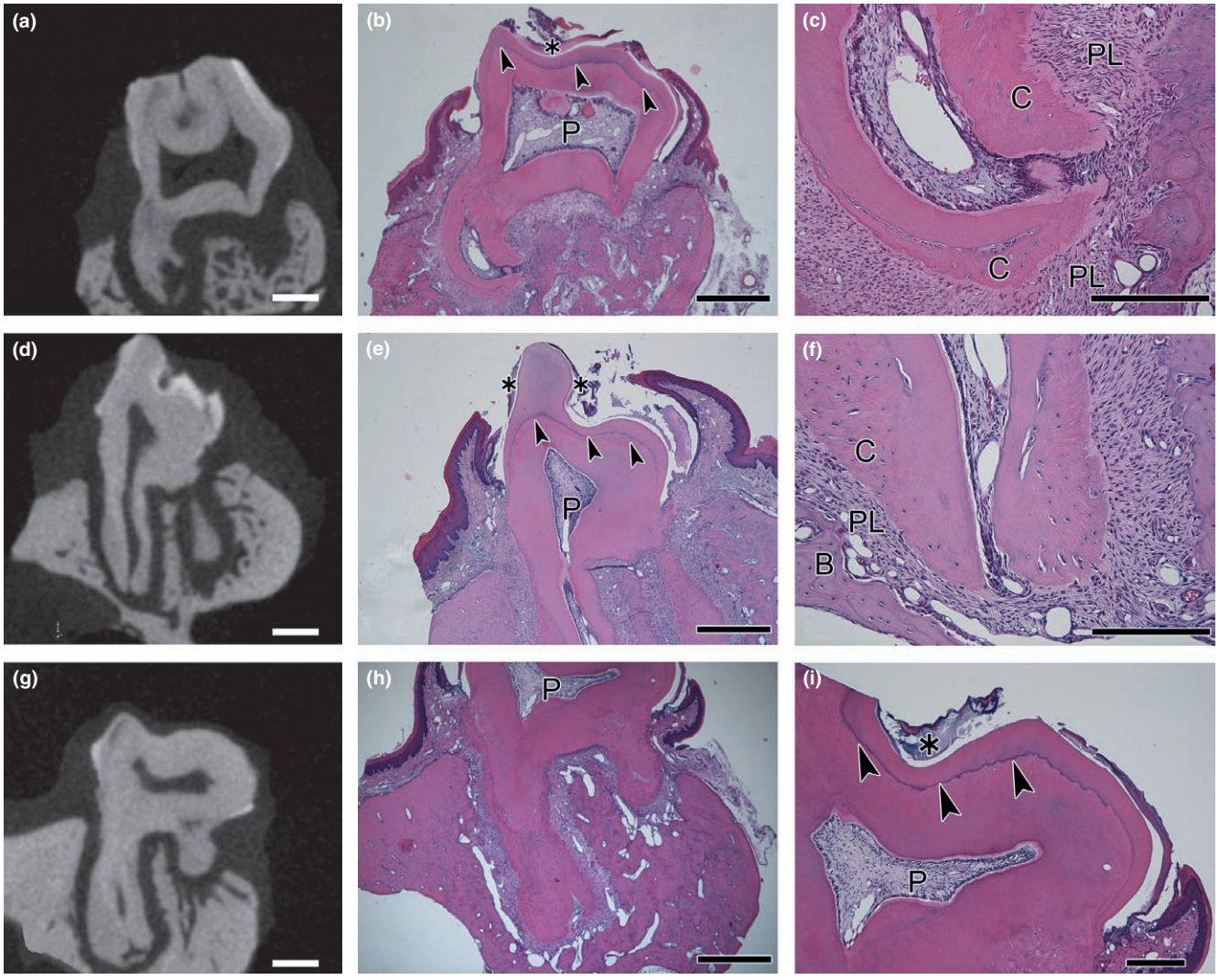

Figure 6 Micro-computed tomography (micro-CT) images (**a**, **d**, **g**) and histology (**b**, **c**, **e**, **f**, **h**, **i**) of transplanted teeth at weeks 4 (**a**–**c**), 8, (**d**–**f**) and 12 (**g**–**i**). Histologic images correspond to those obtained by micro-CT. Transplanted teeth erupt and roots elongate in week 4, and the enamel matrix remains in the crown (asterisk, **b**). Crown dentin is thicker at weeks 8 (**e**) and 12 (**i**), whereas the enamel matrix still remains. The dentin matrix in the transplanted teeth is divided into two layers by a line deeply stained with hematoxylin (**b**, **e**, **i**). Cementum (C), surrounding periodontal ligaments (PL) and alveolar bone (B) are observed (**c**, **f**, **h**). Scale bars = 500 μm (**a**, **b**, **d**, **e**, **g**, **h**) and 200 μm (**c**, **f**, **i**)

of those defects (Tanaka *et al*, 1990; Tsukamoto-Tanaka *et al*, 2006). It remains unknown whether these features in allogenically transplanted teeth were brought about by immune response and other factors. Further investigation is necessary to analyze how immune response influences the length and number of roots as well as the morphology of teeth.

Enamel matrix was recognized in sections of decalcified specimens of implanted teeth at weeks 4, 8, and 12, whereas no enamel matrix was left in control teeth in the corresponding weeks. Our previous study showed that maturation or mineralization of enamel matrix was retarded in autogenous transplantation as well (Akiba *et al*, 2006). The retardation of enamel maturation may be partly attributed to interruption of blood supply (Akiba *et al*, 2006).

Allogenically transplanted teeth have been reported to cause irreversible root resorption, followed by the loss of the tooth graft (Nordenram, 1982; Schwartz *et al*, 1987). Mouse teeth transplanted into tooth sockets allogenically have shown inflammatory pulp tissue and poor arrangement of periodontal ligaments, and the process often results in immune rejection (Kim *et al*, 2006; Unno *et al*, 2009). This study reports that about one-third of allogenically transplantated teeth were either lost or extensively absorbed or suffered from ankylosis of roots, and about 40% of examined transplanted teeth showed degeneration of dental pulp tissue with or without inflamed periodontal ligaments, possibly because of immune rejection (Kim *et al*, 2006; Unno *et al*, 2009). Further investigation will elucidate how immune response is involved in absorption and/or loss of transplanted teeth as well as the reduction in root numbers and length. Local regulation of the immune response may provide for allogenic tooth transplantation the possibility in clinical use.

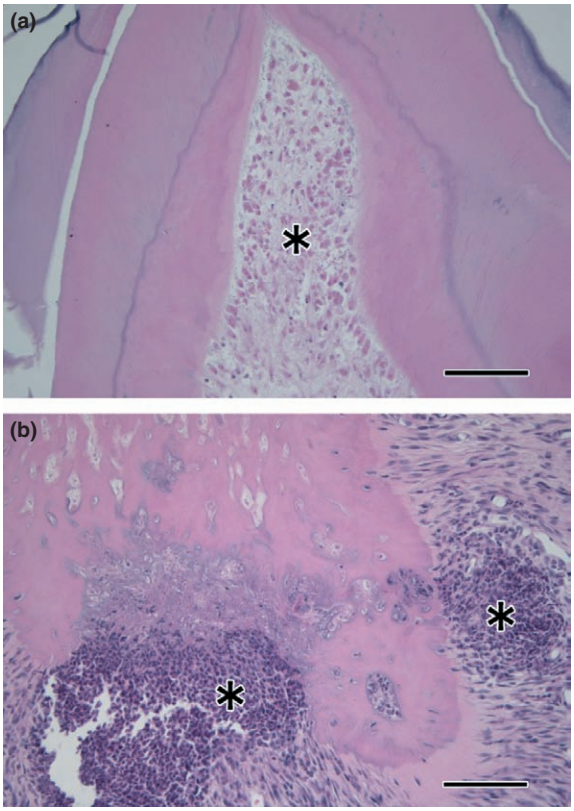

Figure 7 A transplanted tooth in week 4. Dental pulp tissue shows degeneration (**a**, asterisk) accompanied by inflammation in periodontal ligaments (**b**, asterisk). Scale bars = 100 μm

Acknowledgements

This study was supported in part by grants-in-aid (20592134, 20791488) from the Ministry of Education, Science, Sports and Culture of Japan. We thank Mr Yasuto Mikami and Mr Masami Eguchi, Division of Craniofacial Development and Regeneration, Tohoku University Graduate School of Dentistry, for their excellent assistance in this study.

References

Akiba N, Sasano Y, Suzuki O, Sasaki K (2006). Characterization of dentin formed in transplanted rat molars by electron probe microanalysis. *Calcif Tissue Int* **78:** 143–151.

Balto K, White R, Mueller R, Stashenko P (2002). A mouse model of inflammatory root resorption induced by pulpal infection. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **93:** 461–468.

Bauss O, Schwestka-Polly R, Schilke R, Kiliaridis S (2005). Effect of different splinting methods and fixation periods on root development of autotransplanted immature third molars. *J Oral Maxillofac Surg* **63:** 304–310.

Ebina H, Hatakeyama J, Onodera M *et al* (2009). Micro-CT analysis of alveolar bone healing using a rat experimental model of critical-size defects. *Oral Dis* **15:** 273–280.

Ina Y, Sasano Y, Akiba N, Hatori K, Honma T, Sasaki K (2008). Root development of rat tooth germs implanted in the tooth socket and in the subcutaneous tissue. *Oral Dis* **14:** 644–651.

Kim E, Cho SW, Yang JY *et al* (2006). Tooth survival periodontal tissues healing of allogenic-transplanted teeth in the mice. *Oral Dis* **12:** 395–401.

Myrlund S, Stermer EM, Album B, Stenvik A (2004). Root length in transplanted premolars. *Acta Odontol Scand* **62:** 132–136.

Nordenram Å (1982). Allogenic tooth transplantation with an observation time 16 years. Clinical report 32 cases. *Swed Dent J* **6:** 149–156.

Schwartz O, Frederiksen K, Klausen B (1987). Allotransplantation of human teeth. A retrospective study of 73 transplantations over a period of 28 years. *Int J Oral Maxillofac Surg* **16:** 285–301.

Shibata T, Komatsu K, Shimada A *et al* (2004). Effects of alendronate on restoration of biomechanical properties of periodontium in replanted rat molars. *J Periodontal Res* **39:** 405–414.

Tanaka T, Morioka T, Ayasaka N, Jilma T, Kondo T (1990). Endocytosis in odontoclasts and osteoclasts using microperoxidase as a tracer. *J Dent Res* **69:** 883–889.

Tsukamoto-Tanaka H, Ikegame M, Takagi R, Harada H, Ohshima H (2006). Histochemical and immunocytochemical study of hard tissue formation in dental pulp during the healing process in rat molars after tooth replantation. *Cell Tissue Res* **325:** 219–229.

Unno H, Suzuki H, Nakakura-Ohsima K, Tung HS, Ohsima H (2009). Pulpal regeneration following allogenic tooth transplantation into mouse maxilla. *Anat Rec* **292:** 570–579.