Original Article

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Differentiation of Murine Enamel Organ-derived Tissue Stem Cells into Cementoblasts after Transplantation

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Abstract

Purpose: It has been proposed that some enamel organ-derived cells undergo an epithelial-mesenchymal transition (EMT) during tooth development, differentiate into cementoblasts, and make acellular cementum. The primary purpose of this study was to elucidate the mechanisms of cell differentiation by transplanting apical bud-derived stem cells of the epithelial lineage of tooth embryos into the bony defects around the molars and tracking their dynamics. The secondary purpose of this study was to use those tissue stem cells as a source of cell transplantation for future periodontal tissue regeneration therapy.

Methods: Apical bud cells from GFP-positive mouse incisors were isolated under a microscope, collected, dispersed, and transplanted into bone defects created around the molars of wild-type mice, and their dynamics were observed for three months. Undecalcified cryosections were made by Kawamoto's method, and immunofluorescence staining was made using antibodies against Osteocalcin (OCN), Cementum protein 1 (CEMP1), and Cytokeratin 10 (CK10).

Results: GFP-positive cells were observed to settle in the periodontal tissue after four weeks of transplantation. Most of the GFP-positive cells showed OCN positivity throughout the observation period. In 12 weeks, some transplanted cells were aligned along the surface of the cementum, and HE images showed the development of cement matrix-like structures around the cells. Among the transplanted cells, cells in the periodontal space were mostly CK10-positive and CEMP1-negative, while cells on the cementum and near alveolar bone were mostly CEMP1-positive and CK10-negative.

Conclusion: Our observations revealed that some transplanted cells underwent EMT and became cementoblast-like on the root surface and osteoblast-like, where they were incorporated into the alveolar bone. Both cementoblast-like and osteoblast-like cells were CEMP1-positive. On the other hand, CEMP1-negative and CK10-positive cells were located in the middle of the periodontal ligament space, suggesting that they settled into the tissue as ERM without undergoing EMT. It is interesting to see how the transplanted cells migrate and settle or differentiate under the influence of surrounding cells. These results suggest that enamel organ-derived tissue stem cells may differentiate into osteoblasts and cementoblasts/cementocytes and could be the source of periodontal tissue regeneration.

Key words: cementoblasts, epithelial-mesenchymal transition, Hertwig's epithelial root sheath

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Introduction

The lifelong elongation of rodents' maxillary and mandibular incisors has a unique anatomical structure, with enamel forming on the labial side and the periodontal ligament starting on the lingual side. The most apical side of the incisors, called the labial cervical loop initially, has been named the apical bud by Harada et al.[1]. The apical bud contains a cell population composed of epithelial tissue stem cells corresponding to human enamel organs[1–3]. Using the apical bud as a grafting material may be a valuable tool for studying cell differentiation.

We have already reported the successful development of a technique in which incisor apical bud cells from GFP-positive transgenic mice were harvested under a microscope and transplanted into the incisor root tips of wild-type mice[4]. The apical bud cells differentiated into ameloblasts/Hertwig's epithelial root sheaths (HERS)/epithelial cell rests of Malassez (ERM). HERS can stimulate and transform mesenchymal cells of the dental follicle into cementoblasts. HERS eventually remains in the periodontal ligament to form ERM, some of which undergo apoptosis[5,6]. The functions of the ERM remaining in the periodontal ligament are thought to be diverse, including maintenance of the periodontal space, prevention of root resorption and ankylosis, maintenance of homeostasis of the periodontal ligament, and induction of acellular cementum formation[7].

Recently, it has been proposed that HERS undergoes an epithelial-mesenchymal transition (EMT) during tooth development and differentiates into cementoblasts[8–12]. Much circumstantial evidence supports this theory in several *in vitro* experimental systems. However, some studies suggest that there are no or few HERS cells that differentiate into cementoblasts[13–17].

Especially in externally transplanted HERS, it is unclear whether these cells can generate EMT and whether such cell transplantation can be applied for periodontal tissue regeneration with cementum formation.

The primary purpose of this study was to elucidate the mechanisms of cell differentiation by transplanting apical bud-derived stem cells of the epithelial lineage of tooth embryos and tracking their dynamics. The secondary purpose of this study was to use those tissue stem cells as a source of cell transplantation for future periodontal tissue regeneration therapy. In this study, apical bud cells from GFP-positive mouse incisors were isolated under a microscope, collected, dispersed, and transplanted into bone defects created in the molars of wild-type mice. Their fate was observed for three months. Cellular characteristics were monitored using antibodies against Osteocalcin (OCN), Cementum protein 1 (CEMP1), and Cytokeratin 10 (CK10) as markers for calcification, cementum, and epithelial cells, individually.

Materials and Methods

1. Experimental animals

This study was approved by the Fukuoka Dental College Animal Experiment Committee, considering animal welfare and in compliance with the Fukuoka Dental College Animal Experiment Guidelines (Fukuoka Dental College Animal Experiment Approval No. 20007). Experimental animals were bred in the SPF Laboratory at the Animal Center of Fukuoka Dental College and kept on a 12-hour light/dark cycle according to the facility's guidelines. Fifty-four 10-day-old C57BL/6-TG (CAG-EGFP) mice (Green Mouse, SLC, Shizuoka, Japan) transfected with the GFP gene were used as donors[18,19]. Eighteen 6-week-old male wild-type C57BL/6 mice were used as recipients. Donors were housed with their maternal mice.

2. Cell preparation method from donors

Ten-day-old C57BL/6-TG (CAG-EGFP) mice were euthanized by inhalation of isoflurane anesthesia overdose, the mandible was removed under a stereomicroscope, and the incisor teeth, including the root apex, were extracted from the mandible. The teeth were then treated in 37℃ saline containing 1,500 PU/mL of dispase (Dispase, Gibco, Beverly MA, USA) for 20 minutes, and the apical bud portion was isolated from the root apex (Fig. 1). The isolated apical buds were then incubated in 1 mL of cell detachment solution (Accumax, Innovative Cell Technologies, Inc., San Diego, CA, USA) for 10 minutes at room temperature and collected by centrifugation after cell dispersion.

3. Method of transplantation to the recipient

Under general anesthesia with isoflurane, 6-week-old wild-type mice were fitted with a mouth opening

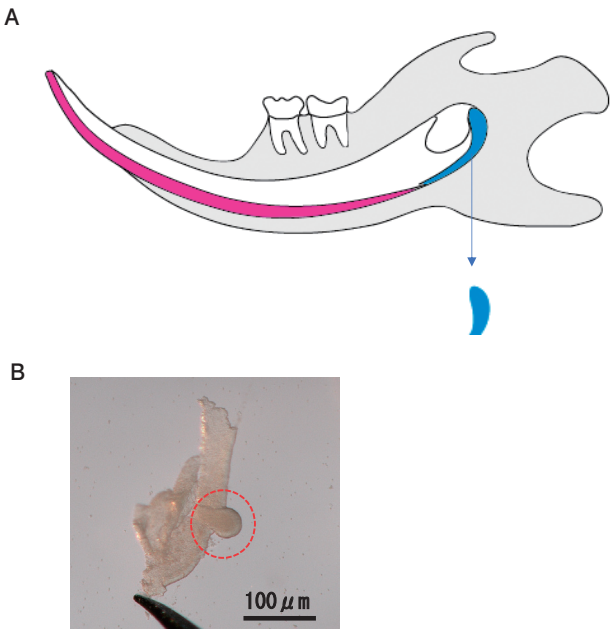

Fig. 1 Schematic illustration of a method for collecting apical buds

A : Sagittal section of mouse mandible. The enamel of the mandibular incisor is shown in red. After removal of the mandibular incisor from the mandible, apical buds and the extending tissue transitioning to enamel epithelium (blue) were harvested.

B : Enamel epithelial tissue containing apical buds collected from the mandibular incisor.
From the tissue fragment shown in the figure, the portion indicated by the dotted circle was separated as an apical bud.

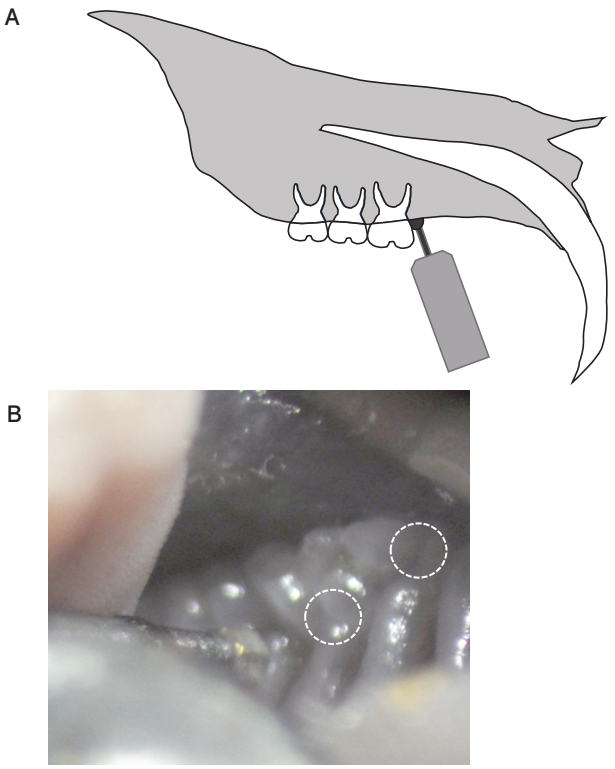

Fig. 2 Schematic illustration of a method for creating bone defects in the recipient

A : Sagittal section model of mouse maxilla. Defects of 0.5 mm in diameter and 1.5 mm in depth were created in two locations on the mesial and palatal sides of the mouse maxillary first molar using a round bar attached to a straight handpiece.

B : The palatal surface of the maxillary first molar undergoing bone defect formation. Drilling was performed at two locations, the mesial surface of the mesial root and the furcation area between the mesial and lingual roots, as indicated by the dotted circles, and cells collected from the donor were carefully injected.

device, and two holes of 0.5 mm in diameter and 1.5 mm in depth were drilled into the periodontal ligament of the maxillary molar using an engine drill without opening periodontal flap (Fig. 2). Compression hemostasis was applied as needed to ensure adequate hemostasis after drilling. The transplantation of cells was performed with a micropipette (Reference 2, Eppendorf, Hamburg, Germany) with a gel loading tip (124-R-204, BIO-BIK, Osaka, Japan). Cell counts were verified on a blood cell counter. An average number of 3.0×10^5 cells were inoculated at one socket. The number of transplanted cells was three μL per defect, and each hole received cells from three donor sites. Dispersed cells were injected into the periodontal ligament and bone defect without carrier materials. No sutures were made after injection. Animals were euthanized at 1, 4, and 12 weeks after transplantation (n=6 per week, a total of 18 animals), and the right and left maxillae were removed and fixed in 4% paraformaldehyde buffer solution.

4. Preparation of pathological specimens

The undecalcified maxillary bone was placed in a 10×10×5 mm cryomold container (Sakura FineTek Cryomold, Osaka, Japan) with the occlusal surface facing upward and frozen in liquid nitrogen with Cryo-Embedding Medium (SCEM, SectionLab, Hiroshima, Japan). The frozen sections were cut with a tungsten knife at −20℃ in a cryostat (CM3050S, Leica, Wetzlar, Germany) at a thickness of 4 μm by the Kawamoto's method (film transfer method) using the height of the root furcation of the maxillary first molar as a refer-

ence. The specimens were observed by HE staining and immunofluorescence staining. For immunostaining, an anti-GFP chicken antibody (Abcam, Cambridge, UK) was used as the primary antibody, and an anti-chicken AlexaFluor 488 antibody (Thermo Fisher Scientific Inc., Cambridge, UK) as the secondary antibody to enhance GFP fluorescence. In addition, six samples at one week, six at four weeks, and three at 12 weeks were treated with anti-OCN antibody followed by anti-rabbit Alexafluor568 antibody. In the three 12-week samples, anti-CEMP1 and anti-CK10 rabbit antibodies (Abcam) were used as primary antibodies and anti-rabbit Alexafluor568 antibody (Thermo Fisher Scientific Inc.) as the secondary antibody. All sections were sealed in VectorShield with DAPI (Vector Laboratories, Newark, CA, USA). Tissue sections were observed with a fluorescence microscope (BZ-X710, Keyence, Osaka, Japan).

Results

Representative examples of tissue images from 1 to 12 weeks after transplanting cells collected from donor mice into the first molar area of recipient mice are shown.

1. One week after transplantation

Images at one week after transplantation are shown in Figure 3. The low-magnification HE image showed drilled bone defects on the mesial surface of the maxillary first molar's mesial root and the lingual root's mesial furcation side. The transplanted cells showed green fluorescence over a wide area on the mesial surface of the maxillary first molar's mesial root and the lingual root's mesial furcation. Most transplanted cells strongly expressed OCN one week after surgery, although the HE images did not show calcification. DAPI images showed that some of the transplanted cells were in an aggregated state.

2. Four weeks after transplantation

Four weeks after transplantation, GFP-positive cells were observed in and around the periodontal ligament (Fig. 4). OCN-positive oval-shaped cells were widely distributed on the cementum and alveolar bone surfaces, and some GFP-positive cells showed strong OCN expression. These cells showed cementoblast-like and osteoblast-like morphology on HE images.

3. Twelve weeks after transplantation

Images at 12 weeks after transplantation are shown

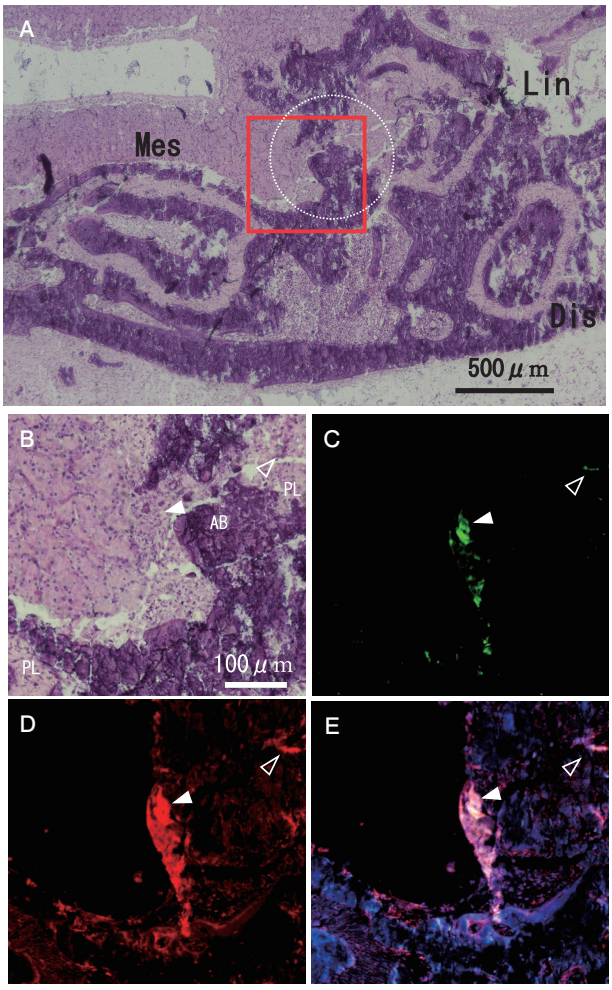

Fig. 3 HE-stained and immunofluorescent-stained images at one week after transplantation

A : HE image of a horizontal section of the maxillary first molar. A magnified image of the red-framed area is shown in B. The area of the created bone defect is shown as a dotted white circle. Mes : mesial root ; Dis : distal root ; Lin : lingual root

B : Magnified HE image of the mesial surface of the lingual root. The arrowhead and outlined arrowhead indicate the transplanted cells. C, D, and E show immunofluorescent-stained images of the same area. AB : alveolar bone ; PL : periodontal ligament

C : GFP-positive cells are mostly found around the alveolar bone (arrowhead) and in the periodontal ligament space (outlined arrowhead).

D : OCN-positive cells are found around the alveolar bone and the periodontal ligament space, overlapping most GFP-positive cells.

E : The merged image of C, D, and DNA stained with DAPI shows that some transplanted cells are in an aggregated state ; most of the GFP-positive cells are OCN-positive.

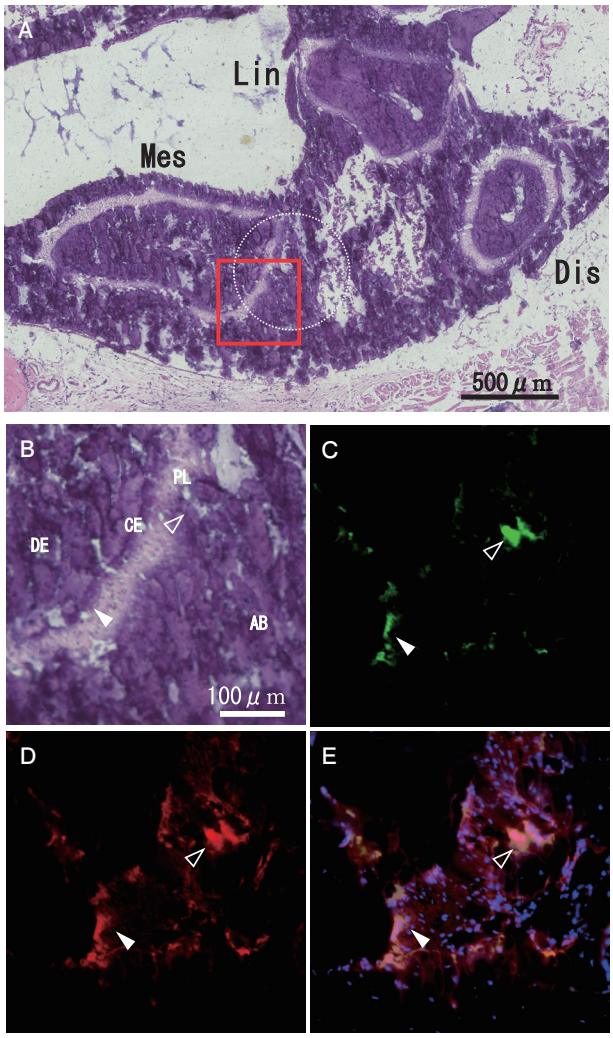

Fig. 4 HE-stained and immunofluorescent-stained images four weeks after transplantation

A : HE image of a horizontal section of the maxillary first molar. A magnified image of the red-framed area is shown in B. The area of the created bone defect is shown as a dotted white circle. Mes : mesial root ; Dis : distal root ; Lin : lingual root

B : Magnified HE image of the distal surface of the mesial root. The arrowhead and outlined arrowhead indicate the transplanted cells. C, D, and E show immunofluorescent-stained images of the same area. AB : alveolar bone ; PL : periodontal ligament ; CE : cementum ; DE : dentin

C : GFP-positive cells are partly aligned along the cementum surface (arrowhead) and partly in the periodontal ligament space close to the alveolar bone (outlined arrowhead).

D : OCN-positive cells are found on the cementum and alveolar bone.

E : Merged image of C, D, and DNA stained with DAPI showing that most of the GFP-positive cells are OCN-positive.

in Figure 5. GFP-positive cells produced cementoblast-like cells in the periodontal ligament and cement matrix-like structures around the partitions on HE images. GFP-positive cells were also found along the cementum surface of the root and on the alveolar bone side across the periodontal ligament, which was OCN-positive. Some GFP-positive cells were also found in the periodontal space, and even though they were OCN-positive, they did not show calcification on the HE images.

4. CEMP1-and CK10-positive cells at 12 weeks after transplantation

Images at 12 weeks after transplantation are shown in Figure 6. Some of the GFP-positive cells migrating into the periodontal ligament were found. In addition, some of the cells were aligned along the surface of the cementum, indicating CEMP1 positivity, and HE images showed the development of cement matrix-like structures around the cells. Cells in the periodontal ligament were mainly CK10-positive and CEMP1-negative, while many CEMP1-positive and CK10-negative cells were found on the cementum and alveolar bone surfaces.

Discussion

In our previous study, we transplanted the apical tissue of the mandibular incisor, including epithelial and mesenchymal cells derived from GFP mice, into wild-type mice and observed the dynamics of the cells by GFP green fluorescence[4]. In the current study, to further limit the number of cells to be transplanted, we performed enzyme treatment on the grafts and transplanted only cells dispersed from the isolated apical buds. With this new approach, we aimed to elucidate the fate of HERS cells after tissue transplantation and to utilize the cells in new cell-based regenerative therapies.

In periodontal tissue regeneration therapy, the cementum, the periodontal ligament, and the alveolar bone are expected to be regenerated. This is because the cementum is calcified periodontal tissue bound to the dentin, and one end of the primary fibers of the periodontal ligament enters the cementum as Sharpy fibers, while the other end joins the alveolar bone to help hold the tooth in place. This study investigated the kinetics of epithelial tissue stem cells after transplantation by tracking GFP fluorescence.

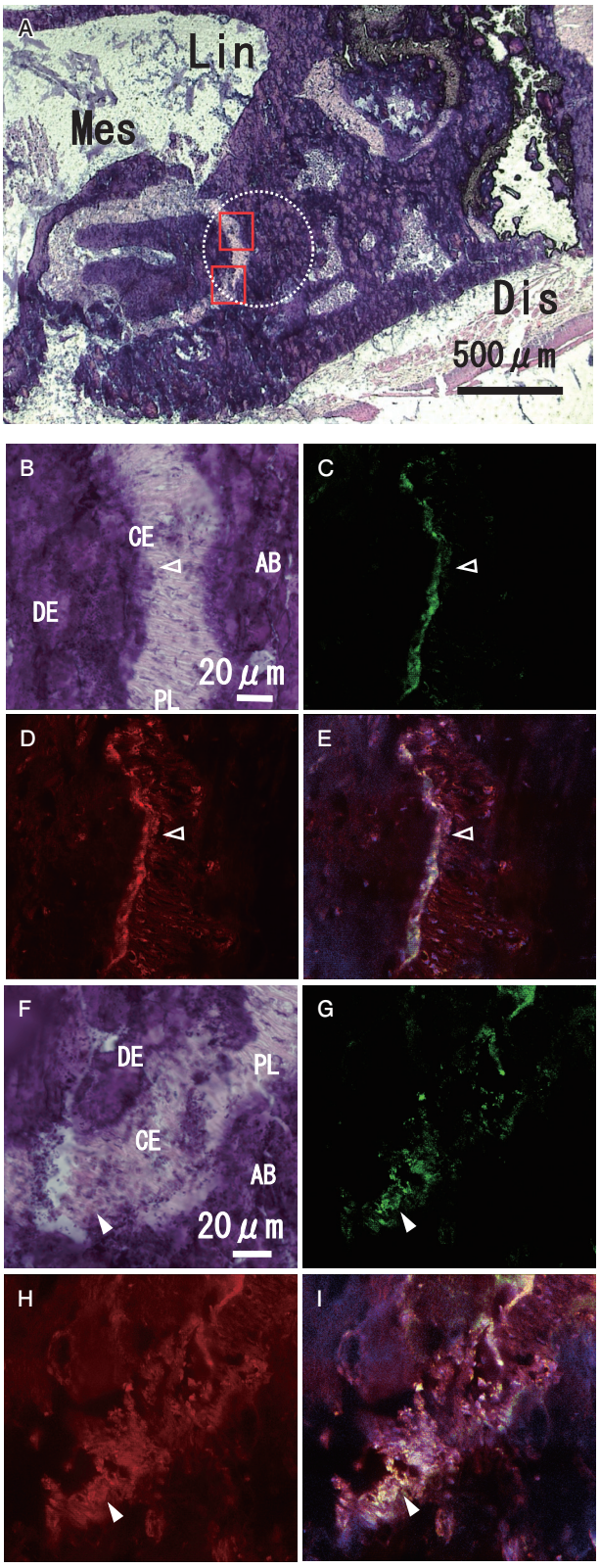

Fig. 5 HE-stained and immunofluorescent-stained images at 12 weeks after transplantation

A : HE image of a horizontal section of the maxillary first molar. Magnified images of the red-framed areas are shown in B and F. The area of the created bone defect is shown as a dotted white circle. Mes : mesial root ; Dis : distal root ; Lin : lingual root

B : Magnified HE image of the distal surface of the mesial root (palatal side). The outlined arrowhead indicates the transplanted cells. C, D, and E show immunofluorescent-stained images of the same area. AB : alveolar bone ; PL : periodontal ligament ; CE : cementum ; DE : dentin

C : GFP-positive cells are aligned along the cementum surface (outlined arrowhead).

D : OCN-positive cells are aligned along the cementum surface (outlined arrowhead).

E : Merged image of C, D, and DNA stained with DAPI showing that most of the GFP-positive cells are OCN-positive.

F : Magnified HE image of the distal surface of the mesial root (buccal side). The arrowhead indicates the transplanted cells. Immunofluorescent-stained images of the same area are shown in G, H, and I. AB : alveolar bone ; PL : periodontal ligament ; CE : cementum ; DE : dentin

G : GFP-positive cells are aligned along the cementum surface ; some are found in the periodontal ligament space (arrowhead).

H : OCN-positive cells are aligned along the cementum surface and in the periodontal ligament space (arrowhead).

I : Merged image of G, H, and DNA stained with DAPI showing that GFP-positive cells in the periodontal ligament space are predominantly OCN-positive.

CEMP1 is explicitly expressed in cementoblasts. It is also weakly expressed in mesenchymal cells such as osteoblasts, osteocytes, cement cells, and fibroblasts[20,21]. The results showed that the cells migrated into the periodontal ligament space and differentiated into periodontal and cementoblast-like cells. Some of the cells

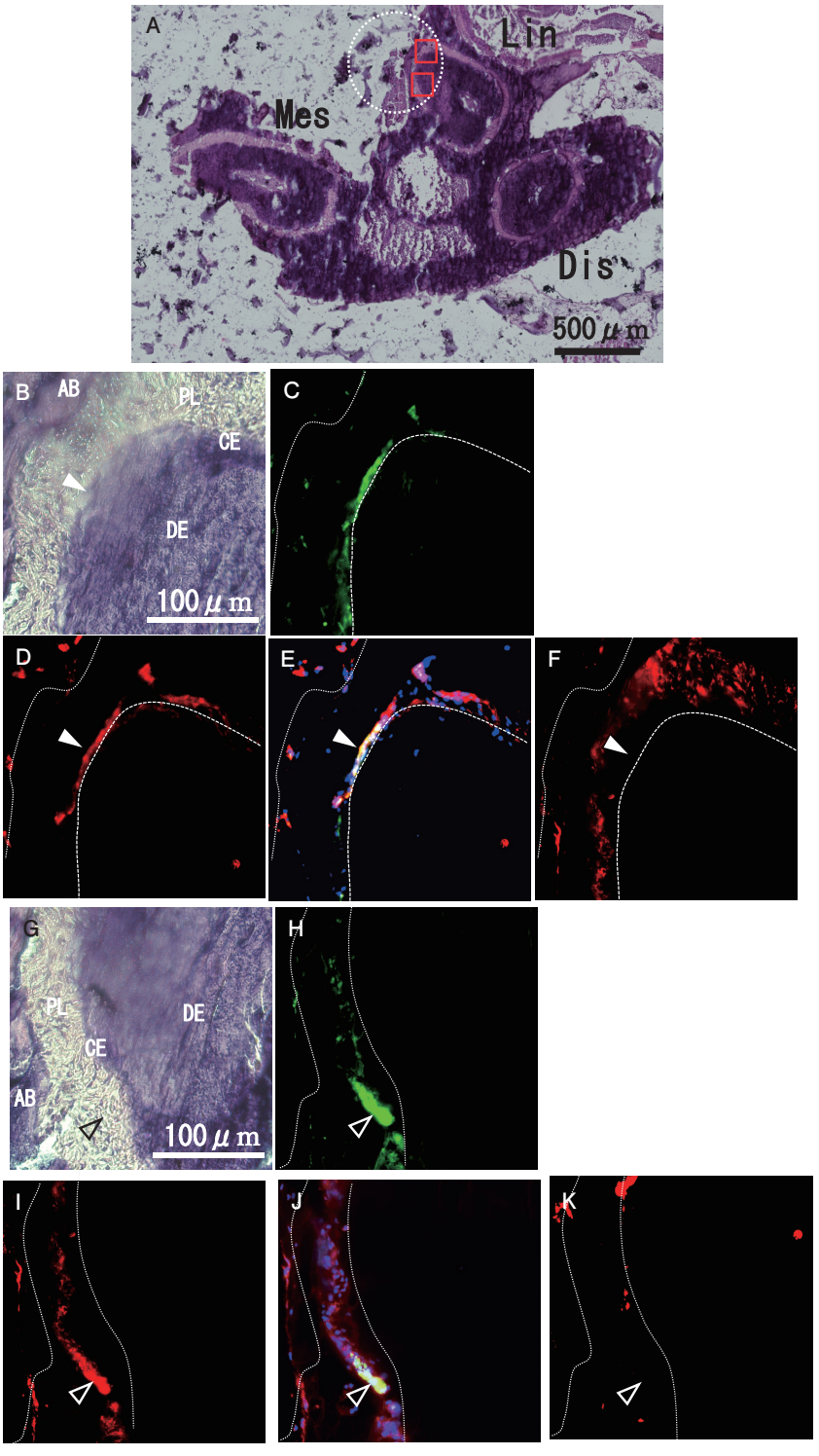

Fig. 6 CEMP1- and CK10-positive cells at 12 weeks after transplantation.

A : HE image of a horizontal section of the maxillary first molar. A magnified image of the red-framed areas is shown in B and G. The location of the created bone defect is shown as a dotted white circle. Mes : mesial root ; Dis : distal root ; Lin : lingual root

B : Magnified HE image of the mesial aspect of the lingual root (palatal side). The arrowhead indicates the transplanted cells. Immunofluorescent-stained images of the same area are shown in C, D, E, and F. AB : alveolar bone ; PL : periodontal ligament ; CE : cementum ; DE : dentin

C : GFP-positive cells are mostly aligned along the cementum surface (arrowhead) and alveolar bone surface.

D : CEMP1-positive cells are found on the cementum and alveolar bone surfaces.

E : Merged image of C, D, and DNA stained with DAPI showing that parts of the GFP-positive cells along the cementum surface are CEMP1-positive.

F : CK10-positive cells are primarily aligned in the periodontal space. CEMP1-positive cells (arrowhead in D) along the cementum surface are found to be CK10-negative in F.

G : Magnified HE image of the mesial aspect of the lingual root (labial side). The outlined arrowhead indicates the transplanted cells. Immunofluorescent-stained images of the same area are shown in H, I, J, and K. AB : alveolar bone ; PL : periodontal ligament ; CE : cementum ; DE : dentin

H : GFP-positive cells are mostly aligned along the cementum surface (outlined arrowhead) and alveolar bone surface.

I : CK10-positive cells are found on the cementum and alveolar bone surfaces.

J : Merged image of H, I, and DNA stained with DAPI showing that among GFP-positive cells, those within the periodontal ligament are predominantly CK10-positive.

K : CEMP1-positive cells are mostly aligned along the cementum surface. CK10-positive cells (outlined arrowhead in I) in the periodontal space are found to be CEMP1-negative in K.

were OCN-positive and CEMP1-positive on the surface of the cementum with cement matrix-like structures.

This experiment observed the healing process in observation periods of 1, 4, and 12 weeks. The transplanted cells were already OCN-positive at one week postoperatively, indicating that a shorter observation period is needed to observe EMT of enamel organ-derived cells. It was deemed necessary to keep the transplanted cells over a long period to determine how they are taken up and function in the tissue.

Our observations revealed that some transplanted cells underwent EMT and became cementoblast-like on the root surface and osteoblast-like, where they were incorporated into the alveolar bone. Both cementoblast-like and osteoblast-like cells were CEMP1-positive. On the other hand, CEMP1-negative and CK10-positive cells were located in the middle of the periodontal ligament space, suggesting that they settled into the tissue as ERM without undergoing EMT. It is interesting to see how the transplanted cells migrate and settle or differentiate under the influence of surrounding cells. These results suggest that enamel organ-derived tissue stem cells can differentiate into osteoblasts and cementoblasts/cementocytes and could be the source of periodontal tissue regeneration.

Numerous *in vitro* and *in vivo* experiments support that HERS undergoes EMT and differentiates into cementoblasts[8–12]. The first report was by Webb et al.[8] in 1996, who showed immunohistochemical co-localization of keratin and vimentin in cementoblasts during acellular cementum formation in rat molars. They assumed that these were HERS-derived and that acellular cementum could be made by HERS-derived cells.

Zeichner-David et al.[9] isolated HERS cells from the molars of genetically modified mice and created HERS-derived cell lines. These cells initially secreted enamel-associated proteins such as ameloblastin but gradually changed morphology to secrete an extracellular matrix with calcification that resembled the structure of acellular cementum. Based on this finding, they hypothesized that acellular cementum is formed from HERS-origin cementoblasts and that cellular cementum is formed from neural crest-origin cementoblasts.

Sonoyama et al.[10] immortalized human HERS cells, established them as cell lines, and investigated their properties *in vitro*. They found that HERS cells could differentiate periodontal ligament stem cells to produce calcified nodules. HERS cells also expressed markers of cementum- and bone-related proteins such as enamel protein, cytokeratin, vimentin, and E-cadherin. Furthermore, TGF-β1-treated HERS cells activated the PI3K/AKT pathway, resulting in EMT, and subcutaneous transplantation of HERS cells after 48 hours of TGF-β1 treatment in mice revealed cementum-like calcification.

Akimoto et al.[11] examined gene and protein expression *in vitro* using HERS-derived cell lines. The results showed that mesenchymal markers such as vimentin and N-cadherin and epithelial stem cell markers such as cytokeratin14, E-cadherin, and p63 were observed. In addition, stimulation with TGF-β captured many mesenchymal markers and EMT markers such as snail1 and snail2. These results indicate that HERS cells may undergo EMT during root formation.

Huang et al.[12] investigated cementum formation in molars by genetically engineering a mechanism by which keratin14-positive HERS cells maintained βgal positivity after EMT in experiments using K14-Cre; R26R mice. They found numerous βgal-positive cells in the cementum near the root apex and concluded that HERS cells had differentiated into cementoblasts. Huang et al.'s study strongly suggests that acellular cementum may be composed of cells derived from the enamel epithelium.

Thus, many studies have shown that HERS cells may differentiate into cementoblasts. It has also been suggested that different mechanisms by cells of other origins may form cellular cementum and acellular cementum.

Furthermore, when we look at the early stages of root development, there is an intermediate cementum at the interface between the dentin and cementum surfaces, produced by cells derived from the enamel epithelium. This suggests that HERS cells can deposit cemental matrix and are likely to differentiate into cementoblasts.

On the other hand, some studies suggest that HERS cells do not become cementoblasts. Although the involvement of HERS in the odontoblast during root development is widely accepted, it is still controversial that HERS cells undergo EMT and differentiate into cementoblasts. Luan et al.[13] used cytokeratin14 as a marker of HERS/ERM cells to observe the root surfaces of mouse molars. They found many cytokeratin14-positive epithelial cells in the cementum, which

they attributed to the encapsulation and incorporation of HERS cells into the calcifying cemental matrix rather than their differentiation into cementoblasts. Although most ERM cells remain close to the cemental surface, they are not strongly involved in cementum formation. Their study supports Lester's view that epithelial cells on the root surface may be bound to the cementum[14].

Hirata and Nakamura[15] reported the absence of keratin-osteopontin co-localization or keratin-bone sialoprotein co-localization in cellular cementum formation using mouse molars. Yamamoto and Takahashi[16] also performed immunohistochemical staining on tissue sections of rat molars and found no evidence of keratin-vimentin co-localization or Runx2-keratin co-localization among cementoblasts and no evidence of epithelial cells differentiating into mesenchymal cells or calcifying[16,17]. The keratin-positive cells in the cementum were not considered cementoblasts/cementocytes, but HERS cells were left behind in the cementum during cementogenesis[12,13]. They concluded that HERS cells do not differentiate into cementoblasts regardless of whether they are in cellular or acellular cementum and that cells in the dental follicle differentiate into cementoblasts, as has been the conventional theory.

As described above, *in vivo* studies have yet to reach a clear conclusion. This is partly due to the limitations of the research methodology. Most *in vivo* studies are based on observations at a single time and do not employ a method to follow cells over time to identify HERS cells. We can conclude from Huang et al[12]. that acellular cementum is produced from HERS cells, at least in mice.

In periodontal tissue regeneration therapy using enamel matrix protein, which is currently used in routine dental practice, the role of enamel organ-derived cells present in the tissue in regeneration is not well understood; Hammaström et al. reported that there is acellular cementum deposition by the combination of enamel matrix proteins in rat molars and monkey anterior teeth[22,23]. They considered that mesenchymal cells differentiated into cementoblasts and formed acellular cementum by the action of enamel matrix protein. However, it has not yet been demonstrated that mesenchymal cells are responsible for developing acellular cementum in periodontal tissue regeneration therapy. It is also possible that the mesenchymal cells themselves are observed as fibroblasts in the tissue due to the EMT of enamel organ-derived epithelial cells.

In this study, animal experiments were conducted in mice to elucidate the role of enamel organ-derived cells in periodontal tissues and to apply them to periodontal tissue regeneration therapy by transplanting them directly into wounds. The cells may have a dual function by becoming cementoblasts and as HERS, inducing surrounding mesenchymal cells to become cementoblasts by releasing enamel matrix proteins.

It has been reported that the transcription factor sox2 regulates apical bud cells[24], and HERS cells express genes related to epithelial stem cells and embryonic stem cell markers such as Oct-4, Nanog, and SSEA-4[25,26], so there is a possibility that these cells can be used for tissue regeneration. However, there is currently no suitable cell source for clinical use, and seeking such a source from extracted teeth, ES cells, or iPS cells, is a high hurdle. However, periodontal tissues have distinct cell populations, such as ERMs, which are essential in cementum homeostasis and repair. There is potential to develop periodontal tissue regeneration therapy using these cell sources[27].

One problem of our study is that the bone defects were too significant compared to those in experimental animals, resulting in excessive invasion. Thus, the results were uneven as a controlled study for tissue regeneration. In the future, we would like to further verify the results by performing cell transplantation with palatal flap formation only, without creating a bone defect.

Conclusion

It was suggested that enamel organ-derived tissue stem cells may differentiate into osteoblasts and cementoblasts/cementocytes and could be the source of periodontal tissue regeneration.

Acknowledgments

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Conflict of Interest

We have no conflicts of interest to disclose regarding the present paper.

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