Tissue-Engineered Odontogenesis

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Abstract: In this study, we have developed a tooth regeneration model derived from mouse tooth germ cells based on tissue engineering. Lower first molars from DDY mice embryos at E 14 were used. Biodegradable Polyglycolate acid (PGA) scaffolds were fabricated, seeded with dissociated the first molars cells, and implanted into the subcapsular layer of kidney in an adult mouse where they allowed to develop over 3, 5, 7, and 14 days. We present histological analyses characterizing tissue-engineered tooth (TE-tooth). Histological analysis of 14 day implants tissue revealed mineralized enamel and dentin layer were apparent, of the kind of found in normally developing teeth.

Key Words: odontogenesis; tissue-engineering; tooth; tooth germ

Introduction

The recent bioengineering of complex tooth structures from postnatal porcine tooth germ cells suggests the potential for the tooth regeneration[1, 2]. Afterward we examined the regeneration process of the TE-tooth with histochemistry and we observed the enamel tissue morphology of the TE-tooth was not identical with that of the natural tooth[3]. On the other hand, previous recombinant experiments have already shown that the dental epithelium has the potential to induce tooth formation prior to the bud stage, whereas this potential shifts to the mesenchyme from the dental epithelium in the mouse embryo. Recently, tooth tissue-engineering based on embryonic cell-tissue re-combination was reported[4]. The technique to regenerate tooth was that the intact embryonic epithelial tissue at E13.5 was combined the reaggregated dental mesenchymal cells[4]. This study suggests that the dental mesenchyme has an ability to produce a tooth, although even dental mesenchyme loses the information from the dental epithelium. Dr. Hu also reported that many tooth germs at the cap stage were dissociated and re-associated the embryonic isolated dental epithelial cells with intact embryonic dental mesenchymal organ and led to create the tooth *in vitro*. This experiment confirmed the role of the cap stage mesenchyme in the control of tooth morphogenesis. However, none of the approaches to produce teeth used dissociated embryo tooth germs cells including the epithelium and mesenchyme. Therefore, this study adopted a tissue engineering system to investigate the capacity of embryo tooth germ cells to produce teeth.

Materials & Methods

The mandibular first molar tooth germs from DDY mice embryos at E14 were dissected free from the surrounding tissue minced. Tissues were enzymatically treated and single-cell suspensions were obtained by passing the cells through a 70-μm strainer. Cells were resuspended at 5.0×10^5 cells/mL and seeded onto PGA to implantation into the kidney capsule of the mouse hosts. Three-dimensional scaffolds (1×1×1 mm) were fabricated from PGA fiber mesh as previously described[3]. Syngeneic male mice of 5 weeks DDY strain mouse were used as recipients. The implants were transplanted into the kidney capsule for 3, 5, 7, and 14 days. The specimens were embedded in paraffin. Then five micron thick specimens were mounted and stained with hematoxylin-eosin. Immunohistochemical analysis was performed with a Vectastain ABC kit. The antibodies used were rabbit anti-mouse collagen type1 polyclonal antibody (1:1000, CALBIOCHEM CO., Darmstadt, Germany).

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Results

Morphological examinations were chronologically performed from the day 3 to the day 14 after implantation. At least 3 experiments with a minimum of 10 mice embryos (20 molar tooth germs) were performed. After 3days implantation, epithelial cells had formed circular aggregates in the presumptive TE-tooth (Fig. 1A). After 5 days implantation, the dental epithelium-like tissue was expanded and corresponds to the shape of dental enamel organ at the cap stage in the natural tooth germ. The epithelial cells that cover the convex surface organized as single layer (Fig. 1B). The expression of collagen type1 can be seen in the condensed dental mesenchymal-like cells (Fig. 1C). After 7 days implantation, the shape of the future TE-tooth was defined and outlined by the junction between line the concavity of the enamel organ-like tissue and the condensed dental mesenchymal-like cells. The dental epithelial invagination which like cervical loop in the natural tooth was observed. The stellate cells lying between the stratum intermedium and outer enamel epithelium compose the stellate reticulum (Fig. 1D). The condensation of the dental mesenchyme increased from 5 days to 7 days (Fig. 1C, D). After 14 days, the TE-tooth had reached the stage when apposition is near complete crown formation. Though the TE-tooth was surrounded by the bone, the root of a tooth and cementum were not observed. The enamel and dentin were more conspicuous, and the crown cusps were prominent. However, the number of crown cusps in the TE-tooth varied with one and two cusps (Fig. 1E). At this period, dentinal tubules, and odontoblasts were found in the high magnification photomicrograph. And row of columnar cells with polarized nuclei were clearly visible. The columnar cells in the TE-tooth were similar to mature ameloblasts in naturally formed mouse tooth germ. The cellular tissue adjacent to the ameloblast like cells was morphologically similar to the stratum intermedium which, in turn, adjoined cellular tissue resembling the stellate reticulum (Fig. 1F).

Discussion

This study is first report to use dissociated embryonic entirely

tooth germ seeded onto PGA scaffold, and infer that E14 tooth germ might contain a subpopulation of undifferentiated cells capable of regenerating the tooth *in vivo*. And the interaction between the epithelium and the mesenchyme in TE-tooth lead that the undifferentiated cells in the tooth germ cells at E14 differentiated into the odontoblast which present adjacent to the forming dentin, and the ameloblast adjacent to the enamel. In previous study, the dental mesenchyme takes the initiative in tooth formation away from the dental epithelium at E14. Therefore, the dissociated embryonic mesenchyme cells must be given the positional information to isolated epithelial cells and may has led to the normal crown morphogenesis in the same way as the previous recombination experiments that the mesenchymal cells of the dental papilla have been shown to regulate tooth shape. Interestingly, the TE-tooth has the normal crown development in the most of specimens. In addition, though the number of crown cusps in the TE-tooth showed one or two cusps, the TE-tooth morphogenesis to early bell stage had a similar process as the natural tooth development, so we are currently investigate the mechanism for modulating cusp patterns. This result in our mouse experiments is unusual in the pig experiments. The realization of difference between the pig experiment and mouse experiment at the tooth achievement will pave the way for tooth regeneration.

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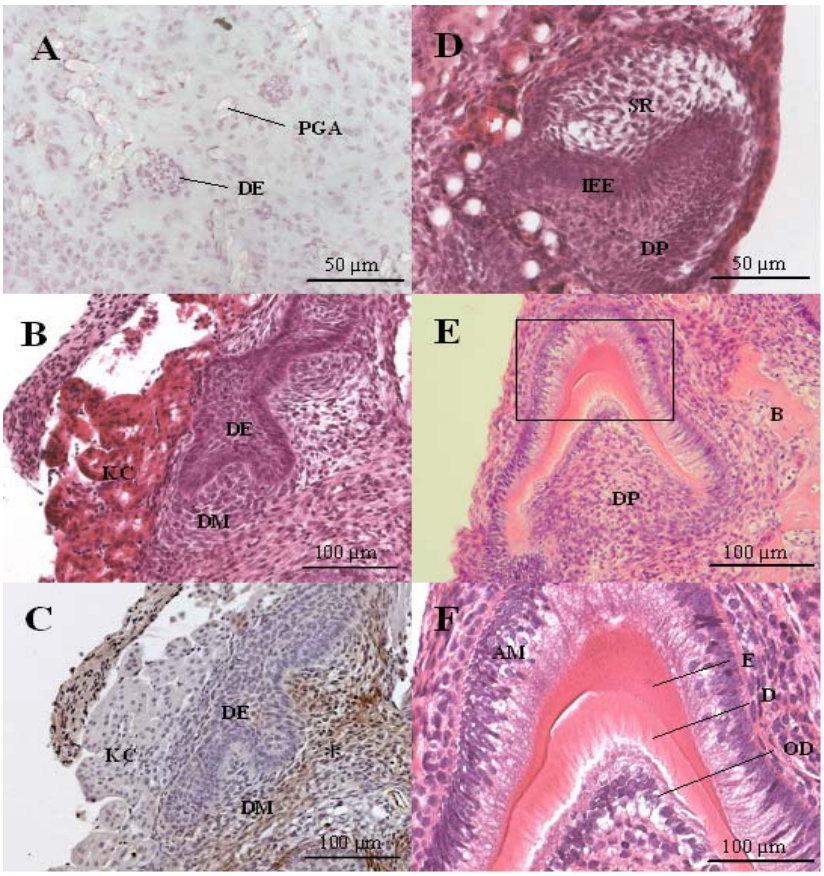

Fig. 1 (A) At 3 days after implantation, reaggregation of the epithelial cells was presented on H&E stained section. Arrowhead shows PGA. (B) At 5 days after implantation, reaggregation of the epithelial tooth bud spheres was presented on H&E stained section. (C) The expression of collagen type 1 was observed in the condensed dental mesenchymal cells at 5 days' implants (asterisk). (D) At 7 days after implantation, the initiation of morphogenesis including the stellate reticulum, inner enamel epithelium, and dental papilla was shown. (E) At 14•@days after implantation, definitive structure of TE tooth was presented on H&E stained section. (F) Enamel, dentin, the epithelial tall columnar cells, and the odontoblast-like cells surrounding the dentin were found at the high magnification photomicrograph. DE: dental epithelium. DM: dental mesenchyme. KC: kidney cortex. SR: stellate reticulum. IEE: inner enamel epithelium. DP: dental papilla. AM: ameloblast. E: enamel. D: dentin. PD: predentin. OD: odontoblast. B: bone.