Stem cells isolated from human dental follicles have osteogenic potential

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Objective. Stem cells isolated from human dental follicles as a potential cell source for bone-tissue engineering were examined for correcting a critical bone defect.
Study design. Impacted third molars were collected and single cell–derived cell populations were cultivated in growth medium. Single cell–derived cell lines were examined in terms of cell shape, gene expression patterns, differentiation capacity in vitro, and osteogenic potential in vivo.
Results. Three distinct cell populations were identified with different morphologies, patterns of gene expression, and differentiation capacity. All 3 cell populations promoted bone formation when transplanted into surgically created critical-size defects in imunodeficient rat calvaria, compared with control animals without cell transplantation, although one of these populations showed a weak capacity for osteogenetic differentiation in vitro.
Conclusions. Human dental follicle can derive at least 3 unique cell populations in culture, all of which promote bone formation in vivo. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:700-708)**

Recently, mesenchymal stem cells (MSCs) were shown to be an attractive cell source for tissue engineering.[1,2] They can be easily isolated from bone marrow (BM) and expanded through several passages while retaining their multipotent differentiation capacity.[3] However, the harvesting of bone marrow is invasive, and many alternative sources of MSCs also have potential in tissue engineering, including adipose tissue[4,5] and dental pulp cells.[6,7] Under appropriate stimulation, MSCs undergo osteogenic differentiation via a well-defined pathway, and bone-tissue engineering using sources such as MSCs is a new tool with the potential to replace autologous tissue grafting for bone defects.[8,9]

Dental follicle is a loose vascular connective tissue composed of a heterogeneous layer of ectomesenchymal cells surrounding the tooth germ in early stages of tooth development.[10-14] In recent years, progenitor cells have been identified in the dental follicle,[15-17] and dental follicle cells have been demonstrated to differentiate along osteogenic pathways.[18-20] We previously identified single cell–derived stem cells in the dental follicle of porcine developing tooth during early crown formation.[21] In that study, the single-cell clonal population combined with β-TCP (β-toricalcium phosphate) formed bone tissue subcutaneously in immunodeficient mice,[21] consistent with other studies.[12,22] More recently, human dental follicle progenitor cells showed hard tissue-forming potential in immunocompromised rats.[23] These results indicate that human dental follicle cells may contribute to bone repair in critical-size defects in vivo. However, no studies have used human dental follicle cells to determine the potential for bone healing, nor has the effect of heterogeneous cell populations derived from human dental follicle tissues on bone repair been addressed.

In the first part of this study, we isolated human dental follicle cells from third molars extracted at the

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crown formation stage. Three distinct populations of dental follicle stem cells were derived with different morphologies, patterns of gene expression, and differentiation capacities. Second, we examined the potential of cell source in 3 distinct populations for bone-tissue engineering in surgically created critical-size defects in imunodeficient rat calvaria.

MATERIALS AND METHODS

The procedures used to acquire all cells from the surgically extracted teeth or from iliac crest conformed to the tenets of the Declaration of Helsinki. This project was approved by the local ethical committee of the Institutional Animal Care and Use Committees (IACUC) at the Institute of Medical Science, the University of Tokyo, and the Ethical Review Committee of the Tokushima University Hospital; all donors gave informed consent.

Isolation of putative human dental follicle stem cells

A total of 3 impacted third molars were collected from 3 healthy patients for orthodontic reasons (18-25 years of age) in the Department of Oral and Maxillofacial Surgery, Tokushima University Hospital. Dental follicle tissue was carefully dissected from the upside of the dental crown and cut into several pieces. These tissue fragments were incubated at 37°C for 30 minutes in a solution containing 0.05% collagenase (Wako, Tokyo, Japan) and 0.125% trypsin (Invitrogen, Life Technologies, Grand Island, NY). After cell populations had adhered to the plastic dish surface, nonadherent cells were removed by change of medium. After cell populations were 80% confluent, cells were suspended at a density of 1 cell per 100 μL and seeded into three 96-well culture plates with the growth culture medium (GCM) consisting of Dulbecco's modified Eagle's medium (DMEM; Kohjin Bio, Saitama, Japan) containing 20% fetal calf serum (FCS; Invitrogen) and 1% antibiotics (Invitrogen). The cells were incubated for 1 week, and then 12 colonies (each from a single cell) were obtained and subsequently expanded in medium containing conditioned medium obtained from the cultured primary dental follicle cells diluted 1:2 in GCM. The cells were gradually passaged from 96-well culture plates into 10-cm dishes for subsequent experiments.

The human dental follicle cells were characterized against control cells isolated from the human periodontal ligament attached to the middle portion of the extracted 3 third molars and from human bone marrow aspirates from the iliac crest (3 patients). These periodontal ligament–derived cells (PDLC) and bone marrow–derived mesenchymal cells (BMSC) were passaged 4 times in GCM and then used in in vitro experiments. Cultured cells were routinely evaluated using phase-contrast inverted microscopy (Olympus Optical Co., Ltd., Tokyo, Japan).

RNA preparation and reverse transcription-polymerase chain reaction

Semiquantitative polymerase chain reaction (PCR) analysis using PDL or osteoblast markers was used to characterize the dental follicle cell populations, PDLC, and BMSC. Total RNA was isolated from each cell population using TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA and amplification was performed in a PCR Thermal Cycler SP (Takara, Ohtsu, Japan) for 25 to 35 cycles according to the following reaction profile: 95°C for 30 seconds, 45 to 60°C for 30 seconds, and 72°C for 30 seconds. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were used as internal standards. Synthesized cDNA served as a template for subsequent PCR amplification using specific primers as listed in Table I. The designed primers were based on the sequence of the target gene. The experiment was performed in triplicate.

Cell growth analysis

To evaluate cell growth, clonal populations (derived from single cells by limiting dilution) of human dental follicle cells were plated at a density of 5×10^3 cells per well and subcultured in GCM for 1, 7, and 14 days in 6-well culture dishes (Becton Dickinson, Franklin Lakes, NJ). Rate of cell growth was calculated by cell counting (Cell-counting Kit-8; Dojindo Laboratories, Kumamoto, Japan) according to the protocol.[24] The relative cell number was determined by measuring absorbance of light at a wavelength of 450 nm (Model 650 Microplate Reader; BioRad Laboratories, Hercules, CA). Results shown are the average of triplicates from one experiment.

Induction of differentiation of dental follicle cells

Three clonal dental follicle cell populations were treated with different media known to induce either osteogenesis, adipogenesis, or chondrogenesis to evaluate their differentiation potential.

Osteogenic differentiation

The osteogenic differentiation capacity of clonal human dental follicle cells was assessed by measurement of ALP activity and staining with alizarin red. The cells were grown on 6-well plates at a density of 5×10^5 cells/well in GCM for 28 days for the assay for ALP activity, and for 14 days for alizarin red staining. For staining of cells with alizarin red, GCM was replaced

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