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Comparison of the bone regeneration ability between stem cells from human exfoliated deciduous teeth, human dental pulp stem cells and human bone marrow mesenchymal stem cells

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ABSTRACT

Cleft lip and palate is the most common congenital anomaly in the orofacial region. Autogenous iliac bone graft, in general, has been employed for closing the bone defect at the alveolar cleft. However, such iliac bone graft provides patients with substantial surgical and psychological invasions. Consequently, development of a less invasive method has been highly anticipated. Stem cells from human exfoliated deciduous teeth (SHED) are a major candidate for playing a significant role in tissue engineering and regenerative medicine. The aim of this study was to elucidate the nature of bone regeneration by SHED as compared to that of human dental pulp stem cells (hDPSCs) and bone marrow mesenchymal stem cells (hBMSCs). The stems cells derived from pulp tissues and bone marrow were transplanted with a polylactic-coglycolic acid barrier membrane as a scaffold, for use in bone regeneration in an artificial bone defect of 4 mm in diameter in the calvaria of immunodeficient mice. Three-dimensional analysis using micro CT and histological evaluation were performed. Degree of bone regeneration with SHED relative to the bone defect was almost equivalent to that with hDPSCs and hBMSCs 12 weeks after transplantation. The ratio of new bone formation relative to the pre-created bone defect was not significantly different among groups with SHED, hDPSCs and hBMSCs. In addition, as a result of histological evaluation, SHED produced the largest osteoid and widely distributed collagen fibers compared to hDPSCs and hBMSCs groups. Thus, SHED transplantation exerted bone regeneration ability sufficient for the repair of bone defect. The present study has demonstrated that SHED is one of the best candidate as a cell source for the reconstruction of alveolar cleft due to the bone regeneration ability with less surgical invasion.

1. Introduction

Stem cells derived from various tissues such as bone marrow, adipose tissue, skin, and umbilical cord have been isolated and examined in terms of cell proliferation and differentiation abilities leading to tissue regeneration. Moreover, in recent years, mesenchymal stem cells (MSCs) derived from dental tissues have been studied due to their higher accessibility regarded as the feature.

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Human dental pulp stem cells (hDPSCs) were first isolated in the year of 2000 [1]. Three years later, stem cells from human exfoliated deciduous teeth (SHED) were isolated [2]. Subsequently, stem cells from periodontal ligament and apical papilla were also isolated and characterized [3,4]. Among them, SHED are derived from the pulp of deciduous teeth, which are clinically and biologically discarded tissues. Thus, SHED are an accessible and promising cell source for tissue regeneration.

Cleft lip and palate is the most common congenital anomaly in the orofacial region. Alveolar bone grafting prior to and during orthodontic treatment are essentially required for most of patients. Autogenous iliac bone grafting is the conventional approach to the closure of bone defects at the alveolar cleft [5]. However, substantial surgical invasion with such complications as hypoesthesia and pain is induced at the donor site after surgery. Furthermore, scarring, hematoma, infection, and fracture of the iliac bone have also been reported [6–9]. Harvesting iliac bone is quite invasive for school-age patients. Consequently, development of a less invasive method has been hopefully anticipated. Toward this goal, we have been developing a certain method available for alveolar bone regeneration for patients with cleft lip and palate using human bone marrow mesenchymal stem cells (hBMSCs). Transplantation of hBMSCs regenerated bone in a dog model of artificial alveolar cleft and an orthodontic tooth movement into the regenerated bone region was achieved [10–12]. However, puncture of the iliac bone is still necessary to obtain cells, and thus, this method still obliges patients an invasion. Therefore, exploring a new cell source for alveolar bone reconstruction is needed.

SHED may be the most promising tool for bone regeneration because of the less invasive procedure for obtaining cells. Application of SHED to regeneration of mineralized tissue have been reported [13–17]. However, comparison of the mineralization ability of SHED, hDPSCs, and hBMSCs has not been fully explored. Moreover, the distribution of cells after transplantation into calvarial bone defects in immunodeficient mice is also still not clear.

The aim of this study was to elucidate the nature of bone regeneration by SHED as compared to that of human dental pulp stem cells and bone marrow mesenchymal stem cells.

2. Materials and methods

Obtaining human dental pulp tissue was approved by the preliminary review board of the epidemiological research committee of Hiroshima University (approval number; E-20). SHED was isolated from upper right primary canine of 11year-old boy who was clinically healthy patients. hDPSCs was isolated from upper right canine of 32-year-old female who was clinically healthy patients and underwent extraction due to orthodontic treatment. Both teeth were obtained with informed consent. SHED and hDPSCs were isolated and cultured as previously described [1,2]. SHED was from hBMSCs (Lonza Walkersville Inc., Walkersville, MD, USA) were purchased and cultured according to the manufacturer's instructions.

2.1. Multipotency evaluation

2.1.1. Osteogenic differentiation

The isolated cells were seeded at a density of 7.4×10^3 cells/well in a 24-well plate. The cells were grown to approximately 60% confluency, and then the media in the wells were replaced with osteogenic differentiation medium to induce osteogenesis. Mineralization was induced on 80% confluent monolayers by addition of DMEM (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum, 100 U/mL Penicillin (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), 100 μg/mL Kanamycin (Meiji Seika Pharma Co., Ltd.), 0.25 μg/mL Amphotericin (MP Biomedicals, Strasbourg, France), 10^{-7} M Dexamethasone (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich), and 50 μg/mL ascorbate 2-phosphate (MP Biomedicals). Cells were incubated at 37 °C in a 5% CO_2 incubator for 14 days, and the medium was changed every 3 days.

2.1.2. Adipogenic differentiation

When the cells became 100% confluent, the medium was replaced with adipogenic supplement containing hydrocortisone, isobutylmethylxanthine, and indomethacin (R&D Systems, Minneapolis, MN, USA). Cells were incubated at 37 °C in a 5% CO_2 incubator for 14 days, and the medium was changed every 3 days.

2.1.3. Chondrogenic differentiation

Cells were isolated from a monolayer culture and then transferred into tubes to allow formation of three-dimensional (3D) aggregates in medium with chondrogenic supplement containing dexamethasone, ascorbate-phosphate, proline, pyruvate, and recombinant transforming growth factor-β3. Cells were incubated at 37 °C in a 5% CO_2 incubator for 14 days, and the medium was changed every 3 days.

2.1.4. Immunofluorescence staining

Immunofluorescence staining was performed using the human MSC functional identification kit (R&D Systems). After 14-day osteogenic and adipogenic differentiation, the differentiated cells were fixed in 4% paraformaldehyde (Nacalai Tesque Inc., Kyoto, Japan) and rinsed twice with 1% bovine serum albumin (BSA) (Sigma-Aldrich) in phosphate-buffered saline. After 14 days, the pellet culture was fixed in 4% paraformaldehyde and embedded in paraffin. The specimen was cut into sections of 5 μm in thickness with a microtome. The sections were mounted on microscope slides (MATSUNAMI Glass Inc., Ltd., Osaka, Japan). Cells and specimens were then blocked with 0.3% Triton-X (Sigma-Aldrich), 1% BSA, and 10% normal donkey serum (R&D Systems) for 45 min. Then, cells and specimens were incubated with mouse anti-human osteocalcin, aggrecan, or fatty acid-binding protein 4 (FABP4) antibodies (R&D Systems) for 1 h, and incubated for 30 min with a secondary goat anti-mouse immunoglobulin antibody (Southern Biotech, Birmingham, AL, USA). After washing the cells and specimens, the slides were observed with a fluorescence microscope (Biozero BZ8100; KEYENCE, Osaka, Japan).

2.2. Analysis of surface epitopes with flow cytometry

Flow cytometric analysis was performed with unsorted SHED and hDPSCs from the 3rd passage to assess the percentage of cells expressing CD29, CD34, CD44, CD73, CD105, CD271 (Becton Dickinson, San Jose, CA, USA), CD146 (Beckman Coulter, Brea, CA, USA), and STRO-1 (BioLegend Inc., San Diego, CA, USA). Mouse isotype antibodies were served as a control. Ten thousand labeled cells were acquired and analyzed using a FACSVerse flow cytometer (Becton Dickinson) running FLOWJO software (TOMY DIGITAL BIOLOGY Co., Ltd., Tokyo, Japan).

2.3. Cell transplantation

A critical-sized defect of 4.0 mm in diameter was created in the center of the calvaria of immunodeficient mice (BLAB/c-nu: Charles River International Laboratories Inc., Yokohama, Japan) using a trephine bur (IMPLATEX CO., Ltd., Tokyo, Japan) under general anesthesia. These mice were selected to avoid potential immunogenic and graft-rejection responses, because SHED, hDPSCs, and hBMSCs are of human origin. Each cell type was seeded onto a polylactic-*co*-glycolic acid (PLGA) membrane of 4.0 mm in diameter