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Comparative characterization of stem cells from human exfoliated deciduous teeth, dental pulp, and bone marrow–derived mesenchymal stem cells

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ABSTRACT

Objectives: Mesenchymal stem cells (MSCs) are used clinically in tissue engineering and regenerative medicine. The proliferation and osteogenic differentiation potential of MSCs vary according to factors such as tissue source and cell population heterogeneity. Dental tissue has received attention as an easily accessible source of high-quality stem cells. In this study, we compared the in vitro characteristics of dental pulp stem cells from deciduous teeth (SHED), human dental pulp stem cells (hDPSCs), and human bone marrow mesenchymal stem cells (hBMSCs).
Materials and methods: SEHD and hDPSCs were isolated from dental pulp and analyzed in comparison with human bone marrow (hBM)MSCs. Proliferative capacity of cultured cells was analyzed using a bromodeoxyuridine immunoassay and cell counting. Alkaline phosphatase (ALP) levels were monitored to assess osteogenic differentiation. Mineralization was evaluated by alizarin red staining. Levels of bone marker mRNA were examined by real-time PCR analysis.
Results: SHED were highly proliferative compared with hDPSCs and hBMSCs. SHED, hDPSCs, and hBMSCs exhibited dark alizarin red staining on day 21 after induction of osteogenic differentiation, and staining of hBMSCs was significantly higher than that of SHED and hDPSCs by spectrophotometry. ALP staining was stronger in hBMSCs compared with SHED and hDPSCs, and ALP activity was significantly higher in hBMSCs compared with SHED or hDPSCs. SHED showed significantly higher expression of the Runx2 and ALP genes compared with hBMSCs, based on real-time PCR analysis. In bFGF, SHED showed significantly higher expression of the basic fibroblast growth factor (bFGF) gene compared with hDPSCs and hBMSCs.
Conclusion: SHED exhibited higher proliferative activity and levels of bFGF and BMP-2 gene expression compared with BMMSCs and DPSCs. The ease of harvesting cells and ability to avoid invasive surgical procedures suggest that SHED may be a useful cell source for application in bone regeneration treatments.

1. Introduction

Autologous and allogeneic bone transplantation is now the gold-standard treatment for repairing bone defects caused by various diseases, congenital defects, infections, and trauma [1]. However, bone grafting techniques have several disadvantages, including postoperative pain, donor site morbidity, infection risk, and immunologic problems, underscoring the need for appropriate bone substitutes that mimic the osteogenic potential of autologous bone [2]. In recent years, regenerative therapy using undifferentiated mesenchymal stem cells (MSCs) has been actively studied as a way to achieve bone regeneration while avoiding invasive surgical procedures.

The concepts of tissue engineering, as proposed by Langer and Vacanti in 1993 [3], are based on three factors: regenerative cells, cell scaffolds, and bioactive substances. Cells that are capable of regeneration are known as stem cells, and those from bone marrow tissue were first described in 1970 [4]. In particular, bone marrow–derived undifferentiated mesenchymal stem cells

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(BMSCs) are present in very low numbers, constituting 0.001–0.01% of the cellular components of bone marrow. However, these cells can be cultured in a manner that retains their capability to differentiate into a variety of cells after separation from the bone marrow. Therefore, they are expected to be useful in regenerative medicine applications for treating a variety of tissue defects.

Previously, we attempted a bone regeneration therapy technique using BMSCs in a Beagle dog cleft jaw model to achieve jaw regeneration and verify that the teeth can be moved to the regeneration site [5,6]. However, although the bioinvasiveness is greatly reduced using this technique compared with conventional methods, bone marrow aspirate must be acquired to obtain bone marrow fluid, thus not completely eliminating the burden on the patient.

To overcome this disadvantage, we focused on MSCs derived from the dental pulp of the deciduous teeth (human dental pulp stem cells; hDPSCs), the isolation and culture of which from the pulp of the permanent teeth was reported by Gronthos et al., in 2000 [7]. We also used other MSCs derived from the pulp of the deciduous teeth (stem cells from human exfoliated deciduous teeth; SHED), the isolation and culture of which from the pulp of the deciduous teeth was reported by Miura et al., in 2003 [8].

These are more proliferative than human BMSCs (hBMSCs) and are also reportedly capable of differentiating into osteoblasts, adipocyte chondrocytes, and neurons [9,10]. Bone regeneration has been observed in vivo [11]. In addition, hDPSCs and SHED are expected to be useful in bone regeneration therapy because they are more readily obtainable than hBMSCs.

Recently, we compared hDPSCs and hBMSCs as scaffolds using SHED implanted with poly (lactate-co-glycolate) barrier membranes for use in bone regeneration treatment for skull coronary defects in immunocompromised mice. SHED transplantation gave good results in terms of induction of regenerative bone to repair the skull coronary defects in immunocompromised mice, but the percentage of newly formed bone did not differ significantly between hDPSCs and hBMSCs. Histologic analyses showed that SHED were associated with larger osteoid and collagen fiber regions than the other cell types. However, differences in the proliferative and bone differentiation capabilities between SHED, hDPSCs, and hBMSCs in vitro have not been fully elucidated. Therefore, the objective of this study was to compare and investigate the bone regeneration potential of hDPSCs and hBMSCs in vitro, specifically focusing on SHED.

2. Materials and methods

2.1. Cell culture

Human dental pulp tissue was obtained from clinically healthy patients who underwent tooth extraction during orthodontic treatment at Hiroshima University Hospital. The preliminary review board of the Epidemiologic Research Committee of Hiroshima University approved obtaining SHED and hDPSCs (approval no. E-20). SHED and hDPSCs were isolated and cultured as previously described. hBMSCs were obtained from Lonza Walkersville Inc. (Walkersville, MD, USA) and cultured according to the manufacturer's instructions. Oral cleaning and disinfection were performed prior to removal of SHED and hDPSC isolation and culture teeth. Pulp was obtained from exfoliated deciduous teeth that were collected from upper right primary canine of 11-year-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. After removal, the teeth were soaked in phosphate-buffered saline (PBS) (LSI Medience, Tokyo, Japan) containing 100 mM amphotericin (Meiji Seika Pharma, Ltd., Tokyo, Japan). After disinfection with Isodine (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) and Hibiten (Dainippon Sumitomo Pharmaceuticals, Osaka, Japan), the periodontal ligaments were removed as much as possible using a scalpel. The tooth from which the periodontal ligament was removed was then disinfected again and split with an osteoclamp (Summer Corp., Tokyo, Japan) at the cement enamel junction. Pulp tissue was collected and immersed in a collagenase dispase solution consisting of α-MEM (Sigma-Aldrich, St. Louis, MO, USA), 4 mg/ml collagenase (Thermo Fisher Scientific, Waltham, MA, USA), and 3 mg/ml dispase (Joint Spirit Co., Ltd., Tokyo, Japan), and the pulp tissue was cut away using a scalpel. Adequately fragmented pulp tissue was transferred into 10-ml tubes containing collagenase dispase solution and incubated in at 37 °C and 5% CO_2 for approximately 50 min with shaking.

After treatment with collagenase, cell aggregates were removed using 70-μm cell strainers (Corning, NY, USA). The filtered solutions were diluted with medium and then centrifuged at 1500 rpm for 5 min. The supernatant was aspirated and suspended in α-MEM containing 20% fetal bovine serum (FBS) (Daiichi Chemical, Tokyo, Japan), 0.24 μl/ml kanamycin, 0.5 μl/ml penicillin, and 1 μl/ml amphotericin, plated in a calibrated 35-mm cell culture dish (Corning), and incubated under 37 °C and 5% CO_2 vapor phase conditions.

When more than 200 colony-forming cells were present, the cells were removed from the culture dishes using PBS containing 0.25% trypsin (Nacalai Tesque, Kyoto, Japan) and 1 mM EDTA (Wako Pure Chemical Industries, Ltd., Osaka) and then recovered and passaged. After first passage (P1), cultures were incubated under 37 °C and 5% CO_2 vapor phase conditions in 10% FBS/α-MEM containing the antibiotics listed above. Poietics™ human BMMSCs (Lonza, Basel, Switzerland) were isolated from tissue donated from a 51-year-old Oriental female after obtaining permission for their use in research via informed consent/legal authorization. These cells were used in the study from passages 3 to 7.

2.2. Characteristics of SHED, hDPSCs, and hBMSCs

Isolated SHED and hDPSCs and purchased hBMSCs (Lonza Japan Co., Ltd., Tokyo, Japan) were used to compare and investigate changes in expression of target genes induced by cell proliferation, bone differentiation, and bone differentiation, respectively.

2.3. Proliferative capacity of SHED, hDPSCs, and hBMSCs

The proliferative capacity of SHED, hDPSCs, and hBMSCs was investigated based on the following parameters.

2.4. Cellular growth curves and population doubling time comparisons

Proliferative capacity of SHED, hDPSCs, and hBMSCs was evaluated based on cellular growth curves and population doubling time (PDT). A total of 1.0×10^4 P4 cells were seeded in a 24-well plate and incubated under 37 °C and 5% CO_2 vapor phase conditions. Live cells were counted daily through day 10 of culture, and cell growth curves were prepared. PDT was calculated using the following formula:

$$PDT = (t - t0)\log 2/\log N - \log N0\ t,$$

Where t0 indicates time counted for cell count and number of cells at N, N0:t, t0.

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