Int. J. Oral Maxillofac. Surg. 2016; 45: 124–131
http://dx.doi.org/10.1016/j.ijom.2015.06.022, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Research Paper
Stem Cells

Comparison of human mesenchymal stem cells derived from bone marrow, synovial fluid, adult dental pulp, and exfoliated deciduous tooth pulp

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Y. Isobe, N. Koyama, K. Nakao, K. Osawa, M. Ikeno, S. Yamanaka, Y. Okubo, K. Fujimura, K. Bessho: Comparison of human mesenchymal stem cells derived from bone marrow, synovial fluid, adult dental pulp, and exfoliated deciduous tooth pulp. Int. J. Oral Maxillofac. Surg. 2016; 45: 124–131. © 2015 International Association of Oral and Maxillofacial Surgeons. Published by Elsevier Ltd. All rights reserved.

Abstract. Populations of pluripotent stem cells were isolated from bone marrow, synovial fluid, adult dental pulp, and exfoliated deciduous teeth and their multipotentiality properties compared. Osteogenic, chondrogenic, adipogenic, and neurogenic differentiation potentials were examined. Bone marrow mesenchymal stem cells (BMMSCs) and synovial fluid-derived cells (SFCs) showed the highest levels of osteogenesis as expressed by alkaline phosphatase (ALP) activity (0.54 ± 0.094 U/mg protein and 0.57 ± 0.039 U/mg protein, respectively; $P = 0.60$) and by osteocalcin (BGLAP; determined by real-time RT-PCR). SFCs showed the highest levels of chondrogenesis as expressed by ALP activity (1.75 ± 0.097 U/mg protein) and of COL2A1 and COL10A1 by real-time PCR. In terms of adipogenesis, lipid vesicles were observed in the BMMSCs and SFCs. Dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) exhibited neurogenesis potential, as shown by increases in expression of class III β-tubulin (TUBB3) and microtubule-associated protein 2 (MAP2) on RT-PCR. Variability was found in the differentiation potential corresponding to the tendency of the original tissue to differentiate. It is suggested that the cell type should be selected depending on the regenerative treatment regimen.

Key words: differentiation; mesenchymal stem cell; dental pulp; synovial fluid; bone marrow.

Accepted for publication 29 June 2015
Available online 30 July 2015

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Mesenchymal stem cells (MSCs) are considered to play important roles in development, postnatal growth, repair, regeneration, and homeostasis. They have attracted much interest with regard to possible clinical use because of their self-renewing potential and multipotentiality.[1] Autologous transplantation of adipose-derived MSCs, periosteum-derived MSCs, and bone marrow-derived MSCs following *ex vivo* cell expansion shows similar efficacy in the guided regeneration of bone defects.[2] While the bone marrow is a well-accepted source of MSCs,[1] several recent reports have identified human MSCs in various other tissues.[3–6] Many reports have confirmed the efficacy of MSCs in regenerative medicine both *in vitro* and *in vivo*. MSCs obtained from bone marrow (BMMSCs) have been used widely in experimental studies, but these are not trouble-free, with problems of accompanying pain and morbidity and limitations of cell number and cell activity.[5] Alternative sources of MSCs have thus been sought over the years.

The selection of suitable cell populations appears crucial to the outcome of *in vivo* experiments with MSCs.[7] This study group has previously investigated pluripotent cells derived from adult dental pulp (dental pulp stem cells, DPSCs), normal exfoliated deciduous teeth (stem cells from human exfoliated deciduous teeth, SHED), and synovial fluid (synovial fluid-derived cells, SFCs) in patients with temporomandibular joint (TMJ) disorders. Specifically, the pluripotentiality of DPSCs, SHED, and SFCs was shown. These are derived from very accessible tissue sources and are capable of providing sufficient cells for potential clinical application.[8,9]

In the present study, distinctive populations of pluripotent cells were isolated from bone marrow, synovial fluid, adult dental pulp, and exfoliated deciduous tooth pulp and their potential for multipotentiality compared. Their ability to undergo osteogenic, chondrogenic, adipogenic, and neurogenic differentiation was also assessed.

Materials and methods

Isolation and culture of human MSCs

All samples were collected in accordance with the guidelines set by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. BMMSCs processed from marrow aspirates obtained from normal adult volunteers aged 22 years were acquired from Lonza, Inc. (Walkersville, MD, USA) and washed in growth medium. Synovial fluid was collected from patients with TMJ disorders and the SFCs were isolated as described previously.[9] Pulp tissues were excised from normal third molars extracted for impaction and from normally exfoliated deciduous teeth. The tissues were minced, digested for 1 h at 37 °C with 0.3% type I collagenase and 0.4% dispase, and passed through a 70-μm filter to yield single-cell suspensions.[8]

All cells were cultured in complete medium consisting of Advanced DMEM (Dulbecco's Modified Eagle Medium; Gibco Invitrogen, Carlsbad, CA, USA), 5% foetal bovine serum (FBS; HyClone, Thermo Fisher Scientific, Rockford IL, USA), 2 mM L-glutamine (Gibco), and 100 U/ml penicillin–100 mg/ml streptomycin (Pen Strep; Gibco) and incubated at 37 °C in 5% CO_2. At 24 h after initial plating, the cultures were washed with phosphate-buffered saline (PBS) to remove non-adherent cells, and fresh medium was added.

Morphology and immunocytochemistry

The cells were plated on chamber slides and cultured for 7 days. The cells were washed with PBS, fixed in 3% paraformaldehyde (PFA) in PBS for 5 min at room temperature, and washed with PBS containing 0.1% Triton X-100. The cells were blocked in PBS (+) containing 2% bovine serum albumin (BSA) for 30 min at room temperature. The cells were then incubated overnight at 4 °C with primary antibodies diluted in blocking solution. The following primary antibodies were used: anti-human STRO-1 (1:100, MAB1038; R&D Systems, Inc., Minneapolis, MN, USA), CD34 (1:200, MS-363-P0; Lab Vision Corporation, Fremont, CA, USA), CD45 (1:200, MS-413-P0; Lab Vision Corporation), neuron-specific nuclear protein (NeuN) (1:100, MAB377; Chemicon, Temecula, CA, USA), and nestin (1:100, MAB1259; R&D Systems, Inc.). The cells were washed with PBS and then incubated with the following secondary antibodies in blocking solution at room temperature and shielded from the light for 2 h: (1) 1:200, 1020-02, FITC conjugated goat anti-mouse IgM (Southern Biotech, Birmingham, Alabama, USA), (2) 1:200, AP127F, goat anti-mouse IgG, Fc, FITC conjugate (Chemicon), and (3) 1:100, 03-18-06, rhodamine-labelled goat anti-mouse IgG (H + L) (KPL, Gaithersburg, MD, USA). The cells were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 1:1000, 71-03-00; KPL). Finally, the slides were washed and cover-slipped with VECTASHIELD Mounting Medium (Vector Laboratories, Inc., Burlingame, CA, USA).[9]

Osteogenesis

For osteocyte differentiation, the cells were plated on 12-well plates and incubated in complete culture medium at 37 °C in 5% CO_2. Upon reaching confluence, the medium was then switched to osteogenic induction medium consisting of αMEM (minimum essential medium alpha; Gibco) containing 15% FBS, 2 mM L-glutamine, 100 μM L-ascorbic acid 2-phosphate (Wako Pure Chemical Industries, Ltd, Osaka, Japan), 10 mM sodium β-glycerophosphate (Wako), 10 nM dexamethasone (Sigma–Aldrich, Milwaukee, WI, USA), and 100 U/ml penicillin–100 μg/ml streptomycin. The medium was renewed every 3 days for 28 days.

Chondrogenesis

For chondrocyte differentiation, a pellet culture system was used. Approximately 2.5×10^5 cells were placed in a 15-ml tube and pelleted into micromasses by centrifugation at $450 \times g$ for 10 min. The pellet was cultured for 14 days in chondrogenic medium, which contained 100 ng/ml recombinant human bone morphogenetic protein 2 (rhBMP-2), in addition to DMEM/F-12 (Gibco) containing 10% FBS, 1× ITS+ Premix (BD Biosciences, San Jose, CA, USA; 6.25 μg/ml bovine insulin, 6.25 μg/ml transferrin, 6.25 ng/ml selenious acid, 1.25 mg/ml BSA, and 5.35 μg/ml linoleic acid), 50 μg/ml L-ascorbic acid 2-phosphate, 100 mg/ml sodium pyruvate (Wako), 100 nM dexamethasone, and 100 U/ml penicillin–100 μg/ml streptomycin.

Adipogenesis

The cells were plated onto six-well plates and cultured in complete medium at 37 °C in 5% CO_2. Quiescent cultures of confluent cells were treated for 3 days with adipogenic induction medium that consisted of αMEM containing 20% FBS, 2 mM L-glutamine, 60 μM indomethacin (Sigma–Aldrich), 100 μM L-ascorbic acid 2-phosphate, 0.5 mM isobutyl methylxanthine (Sigma–Aldrich), 0.5 μM hydrocortisone (Sigma-Aldrich), 10 μg/ml insulin (MP Biomedicals, Irvine, CA, USA), and 100 U/ml penicillin–100 μg/ml streptomycin. The medium was then switched to maintenance medium consisting of αMEM containing 20% FBS, 2 mM L-glutamine, 100 μM L-ascorbic acid 2-phosphate, 10 μg/ml insulin, and 100 U/ml penicillin–100 μg/ml streptomycin for

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