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SHORT COMMUNICATION

Obtainment of mesenchymal stem cells by surgeons

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Summary Biological and clinical interest in mesenchymal stem cells (MSCs) has risen dramatically over the past two decades. This paper introduces the methods we used to obtain bone marrow MSCs by means of locally available materials. Rabbit bone marrow contents were harvested from rabbit femurs. After lysing red blood cells, the fibroblast-like cells adhering to the flask were collected. The obtained cells from Passage 3 were tested for the potential to differentiate into mesenchymal tissues (osteocytes, chondrocytes, and adipocytes). The cells were also tested for the presence of marker proteins of mesenchymal lineages and the absence of marker proteins of hematopoietic lineages. All of the materials we used to induce differentiation and characterization of cells was obtained from local biotechnical companies. The cells obtained showed evidence of proliferation. After *in vitro* correlative induction, the cells showed the presence of calcium deposition, type II collagen formation, and intracellular accumulation of lipid vacuoles. The cells were also proven to have the presence of a marker protein of mesenchymal lineages (CD44) and the absence of marker proteins of hematopoietic lineages (CD14 and CD45). It is concluded that surgeons can easily obtain MSCs using locally available materials. We would like to share our experience with those who are interested in the research of MSCs.

Conflict of interest: No benefits have been or will be received from any commercial party related directly or indirectly to the subject of this manuscript.

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1. Introduction

Mesenchymal stem cells (MSCs) have been thoroughly studied during the past two decades.[1,2] MSCs are multipotent cells that can replicate and have the potential to differentiate into lineages of mesenchymal tissues, including bone, cartilage, and fat.[2] They are also characterized by the presence of a consistent set of marker proteins on their surface and an absence of marker proteins of hematopoietic lineages.[2]

We successfully obtained bone marrow MSCs of rabbits using facilities available in most hospitals and materials easily purchased from biotechnology companies in Taiwan. We present in detail the methods of how we obtained the MSCs and testify their characteristics of differentiation potentials and specific surface markers.

2. Methods

2.1. Isolation and culture of rabbit bone marrow MSCs

Femurs of rabbits were harvested under general anesthesia and sterile conditions. Muscle and the entire connective tissue were detached from the femurs. The ends of the bones were cut away and an 18-gauge needle was inserted into the femoral shafts. The bone marrow of the shafts was extruded by flushing with low glucose Dulbecco's Modified Eagle Medium (DMEM-LG; Gibco-BRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL), 100 U/mL penicillin (Hyclone, Logan, UT, USA), and 100 μg/mL streptomycin (Hyclone) (Fig. 1). Marrow plug suspension was dispersed by pipetting, filtered through a 70-μm mesh nylon filter (Becton Dickinson Biosciences, Bedford, MA, USA), and centrifuged at 400*g* for 5 minutes. The pellet was resuspended in the red blood cell (RBC) lysis buffer (0.154 M NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM EDTA) for 5 minutes to lyse the RBC and centrifuged at 400*g* for 5 minutes. The supernatant was decanted by pipetting. Cells (1×10^7) were seeded in tissue culture plates (100 mm diameter) and incubated at 37°C in 5% CO_2. After 4 days of incubation, the nonadherent cells were removed by replacing the medium; thereafter, the medium was changed twice a week. At 80—90% confluence, cells were harvested using 0.05% trypsin—EDTA (Gibco) for 5—10 minutes at 37°C. The cells were centrifuged at 400*g* for 5 minutes. The resuspended cells were replated at 1.5×10^6 cells per plate. The culture medium was changed twice a week.

2.2. Observation of differentiation potentials of our rabbit MSCs

The obtained rabbit marrow cells from Passage 3 were tested for the potential to differentiate into mesenchymal tissues.

For osteogenic differentiation, the rabbit cells were cultured for 3 weeks in DMEM-LG containing 10% FBS, 50 μg/mL ascorbic acid (Sigma-Aldrich, St Louis, MO, USA), 10 mM β-glycerophosphate (Calbiochem, San Diego, CA, USA), and 10^{-7} M dexamethasone (Sigma-Aldich). Then, the cells were rinsed with phosphate-buffered saline (PBS, pH 7.4) and fixed with 4% formaldehyde in PBS (pH 7.4) at room temperature for 10 minutes. Finally, the cells were incubated with 2% Alizarin Red (Sigma-Aldrich), pH 4.2, at room temperature for 30 minutes.

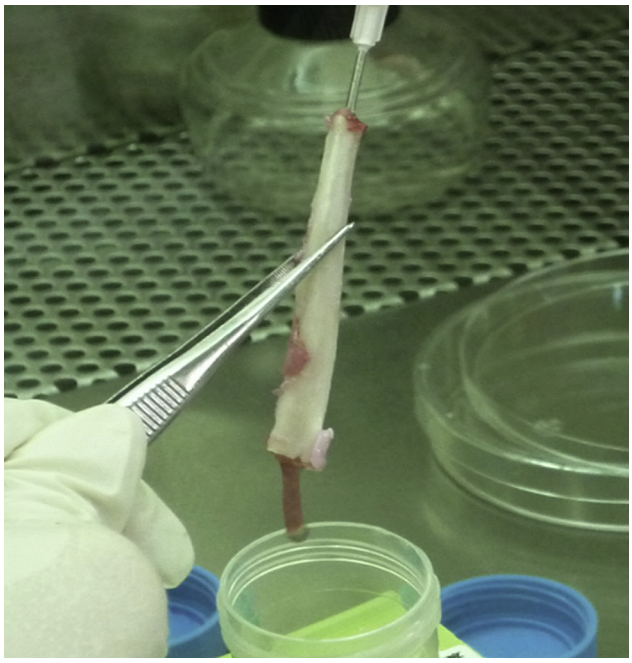

Figure 1 A needle was inserted into the harvested rabbit femur and the marrow of the shaft was extruded by flushing with Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and antibiotics.

For chondrogenic differentiation, the rabbit cells were cultured for 3 weeks in high glucose DMEM (DMEM-HG) containing 1 × insulin-transferrin-sodium selenite (Sigma-Aldrich), 40 μg/mL proline (Sigma-Aldrich), 100 μg/mL sodium pyruvate (Sigma-Aldrich), 50 μg/mL ascorbate-2-phosphoate (Sigma-Aldrich), 10 ng/mL transforming growth factor-β1 (Peprotech, Rocky Hill, NJ, USA), and 10^{-7}M dexamethasone (Sigma-Aldrich). Then, the cells were rinsed with PBS (pH 7.4) and fixed with 4% formaldehyde in PBS (pH 7.4) at room temperature for 10 minutes. Finally, the cells were incubated with Alcian Blue (Sigma-Aldrich) solution for 30 minutes at room temperature.

For adipogenic differentiation, the rabbit cells were cultured for 3 weeks in DMEM-LG containing 10% FBS, 0.5 mM isobutylmethylxanthine (Sigma-Aldrich), 0.2 mM indomethacin (Sigma-Aldrich), 1 μM dexamethasone (Sigma-Aldrich), and 10 μg/mL insulin (Sigma-Aldrich). Then, the cells were rinsed with PBS (pH 7.4) and fixed with 4% formaldehyde in PBS (pH 7.4) at room temperature for 10 minutes. Finally, the cells were incubated with 0.5% Oil Red O (Sigma-Aldrich) solution for 30 minutes at room temperature.

2.3. Fluorescence activated cell sorter analysis and flow cytometry of our rabbit MSCs

Our rabbit MSCs were characterized for the expression of surface markers of MSC using fluorescence activated cell sorter (FACS) analysis with antirabbit CD14, CD44, and CD45 monoclonal antibodies.