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ORIGINAL ARTICLE

An improved protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow

Shuo Huang [a,b], Liangliang Xu [a,b], Yuxin Sun [a,b], Tianyi Wu [a,b], Kuixing Wang [a,b,c], Gang Li [a,b,c,d,e,*]

[a] *Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China*
[b] *Stem Cells and Regenerative Medicine Laboratory, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China*
[c] *Key Laboratory for Regenerative Medicine, Ministry of Education, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China*
[d] *The CUHK-ACC Space Medicine Centre on Health Maintenance of Musculoskeletal System, The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, Hong Kong, China*
[e] *Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China*

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Summary Mesenchymal stem cells (MSCs) from bone marrow are main cell source for tissue repair and engineering, and vehicles of cell-based gene therapy. Unlike other species, mouse bone marrow derived MSCs (BM-MSCs) are difficult to harvest and grow due to the low MSCs yield. We report here a standardised, reliable, and easy-to-perform protocol for isolation and culture of mouse BM-MSCs. There are five main features of this protocol. (1) After flushing bone marrow out of the marrow cavity, we cultured the cells with fat mass without filtering and washing them. Our method is simply keeping the MSCs in their initial niche with minimal disturbance. (2) Our culture medium is not supplemented with any additional growth factor. (3) Our method does not need to separate cells using flow cytometry or immunomagnetic sorting techniques. (4) Our method has been carefully tested in several mouse strains and the results are reproducible. (5) We have optimised this protocol, and list detailed potential problems and trouble-shooting tricks. Using our protocol, the isolated mouse BM-MSCs were strongly positive for CD44 and CD90, negative CD45 and CD31, and exhibited tri-lineage differentiation potentials. Compared with the commonly used protocol, our protocol had higher

* Corresponding author. Room 904, 9/F, Li Ka Shing Institute of Health Sciences, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China.
E-mail address: gangli@cuhk.edu.hk (G. Li).

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success rate of establishing the mouse BM-MSCs in culture. Our protocol may be a simple, reliable, and alternative method for culturing MSCs from mouse bone marrow tissues.

Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells that have the potential to self-renew and differentiate into a variety of specialised cell types such as osteoblasts, chondrocytes, adipocytes, and neurons [1,2]. MSCs are easily accessible, expandable, immunosuppressive and they do not elicit immediate immune responses [3,4]. Therefore, MSCs are an attractive cell source for tissue engineering and vehicles of cell therapy.

MSCs can be isolated from various sources such as adipose tissue, tendon, peripheral blood, and cord blood [5—7]. Bone marrow (BM) is the most common source of MSCs. MSCs have been successfully isolated and characterised from many species including mouse, rat, rabbit, dog, sheep, pig, and human [8—12]. Mice are one of the most commonly used experimental animals in biology and medicine primarily because they are mammals, small, inexpensive, easily maintained, can reproduce quickly, and share a high degree of homology with humans [13]. However, the isolation and purification of MSCs from mouse bone marrow is more difficult than other species due to their heterogeneity and low percentage in the bone marrow [1,14,15].

Two main stem cell populations and their progenies, haematopoietic stem cells and BM-MSCs, are the main residents of bone marrow [1,15]. BM-MSCs are usually isolated and purified through their physical adherence to the plastic cell culture plate [16]. Several techniques have been used to purify or enrich MSCs including antibody-based cell sorting [17], low and high-density culture techniques [18,19], positive and negative selection method [20], frequent medium changes [21], and enzymatic digestion approach [22]. However, they all had some short falls: the standard MSCs culture method based on plastic adherence has been confirmed to have lower successful rate [23]; whereas the cell sorting approach reduced the osteogenic potentials of MSCs [17]. Negative selection method leads to granulocyte—monocyte lineage cells reappearing after 1 week of culture [24]. Cells obtained using a positive selection method show higher proliferation ability compared with the negative selection method, but the method was only repeated in the C57B1/6 mice and failed to repeat in other strains of mice [25]. Frequent medium change method is inconvenient because it is required to change the culture medium every 8 hours during the first 72 hours of the initial culture [21]. Therefore, an easy and effective protocol for isolation of mouse BM-MSCs is needed.

Materials and methods

Reagents

Reagents used included: 0.25% trypsin—EDTA (1×) with phenol red; penicillin—streptomycin— neomycin (PSN; Life Technologies, Carlsbad, CA, USA) antibiotic mixture; foetal bovine serum, qualified, heat-inactivated (Life Technologies); minimal essential medium (MEM) α, nucleosides, powder (Life Technologies); and $NaHCO_3$ (Sigma—Aldrich, St Louis, MO, USA).

Reagent setup

Stock of α-MEM was made up with 1 bag of α-MEM powder (1 L) and 2.2 g $NaHCO_3$ in 1000 mL of Milli-Q water, adjusted to pH 7.2, filtered to sterilise, and stored for 1—2 weeks at 4°C. Complete α-MEM medium was α-MEM medium stock supplemented with 15% foetal bovine serum and 1% PSN, stored at 4°C. Phosphate-buffered saline (PBS) included: NaCl 8.0 g, KCl 0.2 g, KH_2PO_4 0.24 g, and Na_2HPO_4 1.44 g in 1 L Milli-Q water (pH 7.4, sterilised and stored at 4°C).

Animals

In this study, two mouse strains (ICR and C57) with different ages (4 weeks and 8 weeks, males and females) were tested using our protocol. All mice were purchased from and housed in a designated and government approved animal facility at The Chinese University Hong Kong, Hong Kong SAR, China, in according to The Chinese University Hong Kong's animal experimental regulations. All efforts were made to minimise animal suffering.

BM-MSCS culture protocol

Isolation and culture of mouse BM-MSCs

Mice aged 4 weeks or 8 weeks are terminated by cervical dislocation and placed in a 100-mm cell culture dish (Becton Dickinson, Franklin Lakes, NJ, USA), where the whole body is soaked in 70% (v/v) ethanol for 2 minutes, and then the mouse is transferred to a new dish (Fig. 1A). Four claws are dissected at the ankle and carpal joints, and incisions made around the connection between hindlimbs and trunk, forelimbs, and trunk. The whole skin is then removed from the hind limbs and forelimbs by pulling toward the cutting site of the claw. Muscles, ligaments, and tendons are carefully disassociated from tibias, femurs, and humeri using microdissecting scissors and surgical scalpel. Tibias, femurs, and humeri are dissected by cutting at the joints, and the bones are transferred onto sterile gauze. Bones are carefully scrubbed to remove the residual soft tissues (Fig. 1B), and transferred to a 100-mm sterile culture dish with 10 mL complete α-MEM medium on ice (Fig. 1C). All samples are processed within 30 minutes following animal death to ensure high cell viability. The soft tissues are completely dissociated from the bones to avoid contamination.