Original Article

Factors Affecting Mesenchymal Stromal Cells Yield from Bone Marrow Aspiration

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ABSTRACT

Objective: This study was to investigate the variables in bone marrow harvesting procedure and individual donor factors which can potentially affect the yield of mesenchymal stromal cells (MSC).

Methods: We determined the yield of MSC from bone marrow under different clinical conditions by comparing the MSC colony numbers from: (1) donors of different ages; (2) healthy donors and patients with leukemia; (3) bone marrow aspirated at different time points during marrow harvesting; (4) bone marrow harvested by different needles.

Results: During the process of harvesting, the number of MSC significantly decreased with increase number of aspiration, from 675/ml at the initial decreased to 60/ml after 100 ml bone marrow aspirated, and 50/ml after 200 ml bone marrow aspirated. The number of MSC retrieved from leukemia patients (99/ml bone marrow) was significantly lower than that of healthy donors (708/ml bone marrow). However, there was no significant difference in growth rate. There was no significant age-related difference of MSC yielded from donors <55 years. And there was no significant difference in MSC number between the samples from single end-holed needle and those from multiple-side-hole needle.

Conclusion: The optimal bone marrow samples for MSC collection should be obtained earlier in the process of harvesting procedure. Bone marrow from donors <55 years was equally good as MSC sources. The autologous MSC from leukemia patients can be utilized for in-vitro MSC expansion.

Key words: Mesenchymal stromal cell; CFU-F; Bone marrow aspiration; Leukemia

INTRODUCTION

Bone marrow aspiration usually contains mesenchymal stromal cells (MSC) which represent an important cellular component of the bone marrow microenvironment. MSC is a heterogeneous cellular population consisting of mesenchymal stem cells and previous studies showed that the MSC plays important role in supporting hematopoietic stem cells (HSC) engraftment after transplantation[1, 2]. Moreover, recent studies support the potential role of MSC as an immune modulator, and MSC play important role in prevention and treatment of graft-versus-host disease (GVHD)[3-12]. These MSC applied clinically were all in-vitro expanded. How to obtain an adequate amount of MSC within a reasonable limited time frame is crucial for the success of MSC treatment in-vivo. While finding the optimal culture conditions in expanding MSC in vitro is an important issue, how to improve the yield of MSC from proper collection of bone marrow samples is equally vital. This can help to reduce the culture time and chance of contamination, therefore making the expanding process efficient and less costly. Few evidence based data are currently available to guide the clinicians how to select the appropriate technique and donor for the purpose of MSC collection. The current study focused on two specific goals: (1) to determine the variables in bone marrow harvesting procedure which can potentially affect the yield of MSC; (2) to investigate the individual donor factors such as age, gender and disease status which can influence the initial MSC harvest quality.

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MATERIALS AND METHODS

Patients

Bone marrow samples were obtained from 29 healthy bone marrow transplantation donors, and 19 patients with

leukemia. The samples of all leukemic patients are obtained at diagnosis before commencement of treatment. They were diagnosed by standard techniques including morphology, cytochemical staining, immunophenotyping and cytogenetic analysis. The leukemic patients included 6 acute lymphoblastic leukemia (ALL), 6 acute myeloid leukemia (AML), 4 chronic myeloid leukemia (CML), and 3 chronic lymphocytic leukemia (CLL). All bone marrow samples were obtained from the posterior superior iliac crest (PSIC) under either local or general anesthesia. This study was performed with written informed consents under the approval of the Combined Internal Review Board (Ethical Committee) of the University of Hong Kong and The Hong Kong West Cluster of Hospital of Hospital Authority.

Isolation and Culture of MSC

Heparinized bone marrow samples were mixed with 2 volumes of phosphate buffered saline (PBS) and density separated by Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden). Mononuclear cells (MNC) were collected from the interface and washed twice with PBS. The washed cells were resuspended in MSC medium consisting of Dulbecco's modified Eagle's medium-low glucose (DMEM-LG) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mmol/L L-glutamine. The cells were plated at 10^6 cells/100 mm dish. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2. After 24 h, non-adherent cells were removed by changing medium and the plate was washed twice with PBS. Medium was replaced every 3-4 day thereafter. When the culture were near confluence, cells were detached by 0.05% trypsin/25 mmol/L EDTA solution and re-plated at a density of 2×10^5 cells/75 cm^2 flask as first passage. Then the phenotype of the first passage cells was assayed by flow cytometry. The doubling time of MSC was calculated according to the increase of cell number at the end of first passage and the culture time. Selected in vitro expanded MSC from healthy donors and leukemia patients were subjected to immunophenotypic analysis and differentiation testing as we previously reported[13].

MSC Colony Assay

To evaluate the MSC colony (also known as CFU-F, colony forming unit-fibroblast) forming frequency, 10^6 mononuclear cells from bone marrow samples were plated at 100-mm dish in 10 milliliter medium consisting of Dulbecco's modified Eagle's medium-low glucose (DMEM-LG) supplemented with 20% fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mmol/L L-glutamine. The medium was changed after 3 days and then twice a week. After incubation for 14 days at 37°C in 5% humidified CO_2, the cells were washed with PBS and stained with 0.5% Crystal Violet in methanol for 5-10 min at room temperature. After a final rinse in tap water, colonies were counted using a standard inverted microscope. Clusters of cells were considered to be a colony if they contained 50 or more spindle cells. All evaluations were performed in duplicate and expressed as mean values. The frequency of MSC expressed as CFU-F per 10^6 MNC, the number of MSC expressed as CFU-F per milliliter bone marrow.

Effect of Repeated Bone Marrow Aspiration from the Same Donor on the Yield of MSC

To investigate whether the number of MSC was kept at the same level during the whole process of harvesting bone marrow, MSC colony assays were done with bone marrow samples from different aspiration of 20 bone marrow donors. The median age of the donors was 34 years (range from 4 to 55 years). There were 10 male donors and 10 female donors. During the harvest procedure, samples from the first aspiration, the tenth aspiration and the twentieth aspiration from the ipsilateral site of the same donor were collected. We followed the standard practice of changing the puncture sites on the bony surface within the same area of the skin puncture for each aspiration. Each aspiration contained 10 ml of bone marrow; the number of MSC was investigated at the three time points after an estimated amount of marrow was aspirated. The study time points were: (1) at the beginning; (2) after 100 ml of bone marrow was harvested (10th aspiration); and (3) after 200 ml bone marrow was harvested (20th aspiration).

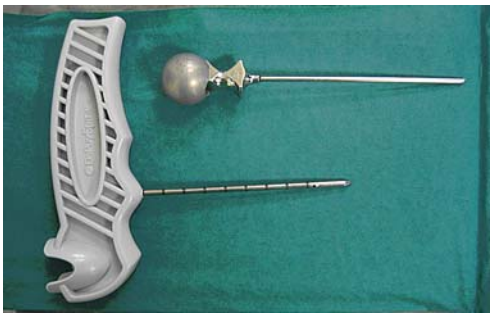

Figure 1. Comparing conventional single end-holed needle (upper) and multiple side-holed DePuy Needle (lower).

Effect of Different Aspiration Needles on the Yield of MSC

We compared the yields of bone marrow derived MSC by using multiple side-holed needle (DePuy Spine 3-hole aspirator needle, DePuy International Ltd., Raynham, Massachusetts, USA) and conventional bone marrow end-holed needle without side-holes (Figure 1). Bone marrow aspiration was performed by both types of needle in a single healthy donor. We performed bone marrow aspirates from the designated bone marrow transplantation donor similar to our routine clinical practice without additional unnecessary punctures. In details, at first, Depuy needle was advanced into the intramedullary cavity at left PSIC, and the conventional needle was advanced into the intramedullary cavity at the right PSIC. Bone marrow 10 ml was aspirated from both sides and 2 ml were saved for analysis, the needles were then removed and the marrow was unloaded into sterile bottles with heparin. Each needle was then again advanced to the contralateral sides. That means Depuy needle to the right and conventional needle to the left PSIC. Another 10 ml of bone marrow were aspirated from each as in the first round and 2 ml each were sent for