The common way of quantifying MSCs is based on the procedure, and it negatively correlates with donor age. With peripheral blood, as well as the aspiration (BMA). It depends on the aspiration site, contamination quality determinant of human bone marrow aspirates. The mesenchymal stromal cell (MSC) content is a major

In previous experiments we have shown that only CD271+ cells, which also express MSCA-1, give rise to CFU-F: MSCs were cultured from 2×10⁷ bone marrow mononuclear cells (BM MNC) using CD271 LAGFR-APC and Anti-APC Microbeads. Samples of all fractions were labeled with CD63-FITC. F1 Fluorescence and light scatter signals were used for gating live cells. The positive fraction contained CD271+ cells with a purity of about 80% (Mean: 83±4.1%; n = 4). This population contained about 73% CD271+CD45− cells (A, orange fraction) and about 13% CD271+CD45− cells (A, red fraction). Further separation of CD271+ and CD271− cells was achieved by flow sorting. The different fractions were cultivated and CFU-F numbers were counted after 14 days. The numbers of CFU-F were about 40-fold higher when MSCs were cultured from the CD271+ compared to CD45− fraction compared to MSCs obtained by plastic adherence (PA). No CFU-F were detected in the CD271− and CD271+CD45− cell fractions (A). Counting of isolated CD271+ cells revealed a 100% co-expression of MSCA-1 in CD271+ cells (B). CD271+CD45−MSCs met ISCT criteria after culture expansion (3 passages) with respect to MSC-specific marker expression (C) and their differentiation potential towards osteocytes, adipocytes, and chondrocytes (D, left to right).

To assess the MSC frequency in human BMA, two samples (MSC sample and control sample) were used. The MSC sample was stained with MSC Staining Cocktail (CD45-FITC, CD271-APC, CD45-APC, and Anti-APC Microbeads). For details on the incubation steps see figure 2. Both samples were analyzed using the same gating strategy (fig. 3):

1: Debris was excluded by gating of leukocytes according to light scatter signals (P1).

2a: Dead cells were excluded according to 7-AAD fluorescence (P2). erythrocytes according to CD235a expression (P3).

2b: Dead cells were excluded according to 7-AAD fluorescence (P2), erythrocytes according to CD235a expression (P3). Addition of FcR

3a: Control sample excluded dead cells (P4).

3b: The sample (MSC sample) was gated on P1, P2, P3 and P4. Addition of FcR

Positive fraction

Before enrichment

CD271label

Negative fraction

Figure 1

Figure 2

Figure 3

4: All CD271+ cells were defined in P4 gated on P1, P2 and P3.

5: Shown is the control sample gated on P1, P2, P3 and P4. MSCs met ISCT criteria after culture expansion (3 passages) with respect to MSC-specific marker expression (C) and their differentiation potential towards osteocytes, adipocytes, and chondrocytes (D, left to right).

The MSC frequency was determined by CFU-F assay and flow cytometry in parallel from 12 bone marrow samples. For this purpose, bone marrow cells were cultivated in a 6-well plate (4×10⁶ cells per well) using StemMACS™ MSC Expansion Media Kit XF. Media was replaced with fresh media after 48 h and colonies were counted after 11 days. Furthermore, CD271+MSCA-1+ cells were quantified using the MSC Enumeration Kit. A linear relationship was observed between the number of colonies and the number of CD271+MSCA-1+ cells in the control sample was below 1% (median = 22; range: 0–88; SD = 30).

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Overall, MSC in the control sample was below 1% (median = 22; range: 0–88; SD = 30).

5a: Control sample

5b: MSC sample

Figure 5

Figure 6

References


