# Isolation of human mesenchymal stem cells: Bone marrow versus umbilical cord blood

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## Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood

**We attempted the isolation and characterization of mesenchymal stem cells (MSCs) from bone marrow (BM) and umbilical cord blood (UBC) in a medium with 10% fetal bovine serum (FBS) and 10% horse serum. In the same conditions it was possible to isolate MSCs from bone marrow but not from UCB.**

In addition to hematopoietic stem cells, bone marrow also contains mesenchymal stem cells[1,2] which contribute to the regeneration of mesenchymal tissues, such as bone cartilage,[3] adipose, muscle, tendon, stroma and neural cells.[3] The existence of mesenchymal stem cells in cord blood is the object of intense discussion.[4-7]

In this study we tried to isolate and characterize MSCs from bone marrow and, using the same culture conditions, from UCB. Thirty-five bone marrow and 58 full-term cord blood samples were harvested. Mononuclear cells were isolated by a Percoll density gradient and cultured in medium with 10% FBS and 10% horse serum. Morphology, immunophenotype and cytokine m-RNA expression were analyzed on adherent cells at every passage. Some cells were cultured under conditions that were favorable for adipogenic, chondrogenic and osteogenic differentiation as described by Pittenger *et al.*[8] Differentiated cells were analyzed by cytochemical staining and by m-RNA specific lineage expression by reverse transcription polymerase chain reaction (RT-PCR). Our results showed that both BM and UCB-derived mononuclear cells, in the same culture conditions, were able to generate an adherent layer. In BM samples the adherent layer was initially formed by individual cells or colonies composed of a few fibroblast-like cells, which rapidly reached confluence and grew exponentially. BM adherent cells were negative for CD45, CD14 and CD34 hematopoietic antigens (Table 1). Conversely, the number and viability of cells in UCB samples rapidly decreased at each passage. The UCB adherent cell immunophenotype analysis was quite different from that of the bone marrow analysis. Indeed, cord blood cells were CD45, CD14, CD31 positive and CD34, CD1a, CD80 negative (Table 1). This contrasts with results from Gutierrez *et al.*[6] who reported that the UCB monolayer contained at least 60% dendritic cells: we found no evidence of dendritic cells in the UCB monolayer. Their culture conditions, however, differed from ours because they isolated the adherent cell population by Ficoll gradient and cultivated them as a dexter long-term culture.

We analyzed the m-RNA expression of two interleukins (IL-6 and IL-11) that are constitutively secreted by MSCs and are stimulators of hematopoiesis[9] and the m-RNA expression of tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and transforming growth factor-$\beta$1 (TGF-$\beta$1) that are pleiotropic cytokines principally produced by monocyte-macrophages. BM adherent cells expressed IL-6 and IL-11 but not TGF-$\beta$1 or TNF-$\alpha$ m-RNA. In contrast, UCB-adherent cells expressed TGF-$\beta$1, TNF-$\alpha$ and IL-6. The absence of IL-11, produced by mesenchymal cells[9] confirmed the absence of MSCs in the UCB samples. TGF-$\beta$1 and TNF-$\alpha$ m-RNA, was probably expressed by monocytes-macrophages present in the adherent UCB monolayer. Moreover, the presence of multi-nucleated cells in UCB adherent cultures that were strongly positive for tartrase-resistant acid phosphatase (TRAP) suggested the spontaneous differentiation of monocytes-macrophages into osteoclasts as described by Erices *et al.*[7]

The differentiation of our cells under specific culture media into the three lineages was demonstrated by cytochemical and molecular analyses (Figure 1). It was not possible to induce mesenchymal differentiation of the UCB monolayer as the cells grown in a specific medium died very quickly.

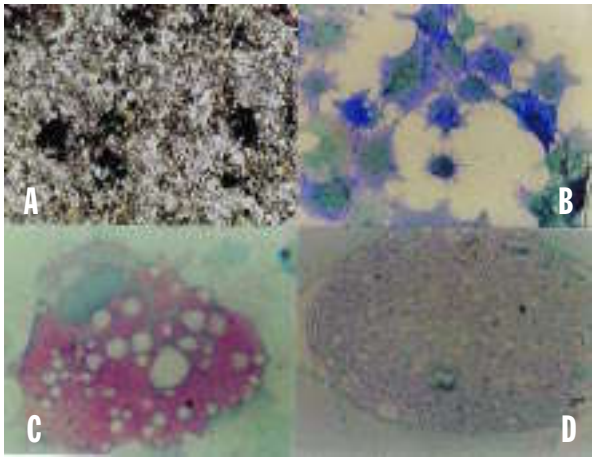


**Figure 1. Photomicrographs showing BM adherent cells induced to differentiate into osteoblasts (A and B), adipocytes (C) and chondrocytes (D). The presence of calcium oxalates seen with Von Kossa staining (A) and the accumulation of intracytoplasmic alkaline phosphatase (B) showed the differentiation of BM MSCs into the osteoblast lineage. Adipogenesis was indicated by the presence of neutral lipid vacuoles that stain with oil red O (C) and chondrogenesis by hyaluronic acid (D: Alcian blue staining) in cells grown as a pellet in the presence of TGF-$\beta$3. Magnification: 20× (A,B,D), 100× (C).**

**Table 1. Immunophenotype analysis of BM and UCB adherent monolayers.**

| *Sample* | *CD45 Median value* | *CD14 Median value* | *CD34 Median value* |
|---|---|---|---|
| BM 1st passage (n=6) | 2.21% (1.20-11.65%) | 1.06 % (0.57-3.48%) | 0.00% (0.00-1.80%)) |
| BM 2nd passage (n=6) | 1.35% (0.00-1.70%) | 0.93 % (range: 0.00-1.70%) | 0.00% (range: 0.00-0.50%)) |
| UCB 1st passage (n=33) | 77.00% (21.00-98.90%) | 17.10 % (0.00-80.00%) | 0.50% (r0.00-2.90%) |
| UCB 2nd passage (n=18) | 87.57% (34.00-98.10%) | 30.50 % (2.90-83.80%) | 0.00% (2.90-83.80%) |

In conclusion, BM contained mesenchymal stem cells that could easily be expanded and induced to differentiate for therapeutic use[3] while the UCB adherent monolayer displayed the morphology and the characteristics of hematopoietic cells and not those of mesenchymal cells. Our data did not agree with the findings of Erices *et al.*[7] who recently identified mesenchymal progenitor cells in 25% of their UCB harvests. It should be pointed out, however, that their results were obtained using a pool of different units of pre-term UCB and, probably, in such a way as to enhance the rather low population of MSCs in pre-term UCB. In 75% of their samples the cord blood adherent cells displayed the morphology and characteristics of multinucleated

osteoclasts, the presence of which agreed with our own findings.

Zvaifler *et al.*[10] recently isolated cells in the elutriatrion fraction of peripheral blood that were able to adhere to plastic and glass and to grow without the addition of growth factors.

Despite our findings, the encouraging results obtained by both Zvaifler *et al.* on peripheral blood and Erices *et al.* on pre-term UCB might be regarded as a further stimulus towards the search for mesenchymal stem cells in UCB by varying isolation and culture conditions.

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