

# The umbilical cord matrix is a better source of mesenchymal stem cells (MSC) than the umbilical cord blood

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## Abstract

Many studies have drawn attention to the emerging role of MSC (mesenchymal stem cells) as a promising population supporting new clinical concepts in cellular therapy. However, the sources from which these cells can be isolated are still under discussion. Whereas BM (bone marrow) is presented as the main source of MSC, despite the invasive procedure related to this source, the possibility of isolating sufficient numbers of these cells from UCB (umbilical cord blood) remains controversial. Here, we present the results of experiments aimed at isolating MSC from UCB, BM and UCM (umbilical cord matrix) using different methods of isolation and various culture media that summarize the main procedures and criteria reported in the literature. Whereas isolation of MSC were successful from BM (10:10) and (UCM) (8:8), only one cord blood sample (1:15) gave rise to MSC using various culture media [DMEM (Dulbecco's modified Eagle's medium) +5% platelet lysate, DMEM+10% FBS (fetal bovine serum), DMEM+10% human UCB serum, MSCGM<sup>®</sup>] and different isolation methods [plastic adherence of total MNC (mononuclear cells), CD3<sup>+</sup>/CD19<sup>+</sup>/CD14<sup>+</sup>/CD38<sup>-</sup>-depleted MNC and CD133<sup>-</sup> or LNGFR<sup>+</sup>-enriched MNC]. MSC from UCM and BM were able to differentiate into adipocytes, osteocytes and hepatocytes. The expansion potential was highest for MSC from UCM. The two cell populations had CD90<sup>+</sup>/CD73<sup>+</sup>/CD105<sup>+</sup> phenotype with the additional expression of SSEA4 and LNGFR for BM MSC. These results clearly exclude UCB from the list of MSC sources for clinical use and propose instead UCM as a rich, non-invasive and abundant source of MSC.

Keywords: cord matrix; mesenchymal stem cell; umbilical cord blood

## 1. Introduction

MSC (mesenchymal stem cells) consist of a rare population of multipotent progenitors having the capacity for self-renewal and differentiation into various lineages of mesenchymal tissues (Bruder et al., 1994). This ability makes MSC an attractive tool in the field of therapeutic use (Tocci and Forte, 2003). Owing to the development of processing and freezing methods for UCB (umbilical cord blood) and the establishment of UCB banks, this material has gained a lot of attention. Although UCB is considered as a rich source of haematopoietic cells (Grewal et al., 2003), the possibility of establishing MSC cultures from this material is still controversial. Whereas some studies clearly failed to establish such cultures (Gutierrez-Rodriguez et al., 2000; Mareschi et al., 2001; Wexler et al., 2003), others using various methods of isolation (Tondreau et al., 2005; Lin et al., 2008; Barachini et al., 2009), culture media (Del et al., 2007; Shetty et al., 2007) and parameters of samples selection (Bieback et al., 2004) succeeded at isolating significant numbers of MSC at sufficient yield for clinical application.

In an attempt to confirm and compare the criteria and the methods used in the literature to isolate MSC from UCB, we carried

out experiments to establish MSC cultures from samples, following parameters optimized in the literature such as a delay from collection to isolation of less than 15 h, a net volume of more than 33 ml with a total MNC (mononuclear cell) count  $>1 \times 10^8$ . We used different culture media [DMEM (Dulbecco's modified Eagle's medium) +5% platelet lysate, DMEM+10% FBS (fetal bovine serum), DMEM+10% human UCB serum or MSCGM<sup>®</sup>] and different methods of isolation such as plastic adherence of MNCs, depletion of CD3<sup>+</sup>/CD19<sup>+</sup>/CD14<sup>+</sup>/CD38<sup>+</sup> cells and selection of CD133<sup>+</sup> or LNGFR<sup>+</sup> cells.

The results showed that the yield of MSC recovery from UCB was far too low to be considered as a reliable source for experimental and clinical use. Instead, we confirmed that UCM (umbilical cord matrix) is a rich source of MSC.

## 2. Materials and methods

### 2.1. Isolation and culture of MNC from bone marrow and UCB

This study was approved by the Ethics Committee of the University of Liège.

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**Abbreviations:** bFGF, basic fibroblast growth factor; BM, bone marrow; DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; FBS, fetal bovine serum; IMDM, Iscove's modified Dulbecco's medium; MNC, mononuclear cell; MSC, mesenchymal stem cells; UCB, umbilical cord blood; UCM, umbilical cord matrix.

BM (bone marrow) cells were obtained from iliac crest marrow aspiration of healthy donors after informed consent. We separated cells on Ficoll density gradient, and the MNC fraction was collected and washed in PBS.

UCB units from full-term deliveries were collected with informed consent of the mothers. We selected only units fulfilling all inclusion and exclusion criteria determined by the UCB bank in accordance to FACT/Netcord standards, but not accepted by the UCB bank because of insufficient cell number for transplant purposes. We separated cells on Ficoll density gradient, and the MNC fraction was collected and washed in PBS. In some experiments, MNC were also enriched for CD133<sup>+</sup> (CD133 antigen, epitope CD133/1, isotype: mouse IgG1 clone AC 133) or LNGFR<sup>+</sup> cells using magnetic beads coupled to antibodies according to the instructions of the manufacturer (Miltenyi Biotec) or depleted of CD3<sup>+</sup>, CD19<sup>+</sup>, CD14<sup>+</sup> and CD38<sup>+</sup> cells (RosetteSep Human Mesenchymal Stem Cell Enrichment Cocktail, StemCell Technologies). The yield of enrichment was monitored by flow cytometry using phycoerythrin-coupled anti-CD133 or anti-LNGFR antibodies (Pharmingen-BD). We seeded  $5 \times 10^5$  cells/cm<sup>2</sup> in six-well plates in various media [DMEM+5% platelet lysate, DMEM+10% FBS, DMEM+10% human UCB serum, MSCGM<sup>®</sup> (Lonza)] in the presence of 100 units/ml penicillin and 100 U/ml streptomycin, at 37°C, in 5% CO<sub>2</sub>. Every 3 to 4 days, we changed the medium until subconfluency was obtained, and cells were harvested with a trypsin-EDTA solution (GibcoBRL) and replated at 1000 cells/cm<sup>2</sup>. Platelet lysates were prepared from PRP (platelet rich-plasma) by several freeze/thaw cycles followed by centrifugation to remove platelet fragments.

## 2.2. Isolation and culture of MSC from UCM

Processing of umbilical cord samples and subsequent isolation of UCM-MSC from the endothelium/subendothelium layer of the human umbilical cord were achieved as follows. Umbilical cords were obtained after Caesarean term deliveries from healthy infants under aseptic conditions and transported to the laboratory in filtered PBS (pH 7.4) containing penicillin (300 units/ml), streptomycin (300 µg/ml), gentamicin (150 µg/ml) and amphotericin B (1 µg/ml), and were processed within 6–12 h. The umbilical veins were washed twice with PBS containing 100 units/ml heparin. The umbilical cord was cut into small pieces and incubated in hyaluronidase (0.5 mg/ml) for 30 min at 37°C and collagenase (0.8 mg/ml) (Sigma-Aldrich) overnight at 37°C in DMEM. After filtration, to remove pieces, and washing in PBS, cells were counted and cultured at  $5 \times 10^5$  cells/cm<sup>2</sup> in DMEM supplemented with 5% platelet lysate at 37°C in 5% CO<sub>2</sub> atmosphere. Non-adherent cells were removed after 3 days by changing the medium, and adherent cells were kept in culture, while being fed with fresh medium every 3 days until the outgrowth of fibroblast-like cells. At confluence, cultures were harvested with PBS (pH 7.4) containing trypsin (0.05%) and passaged into a new flask for further expansion.

## 2.3. Immunophenotyping of MSC

To detect surface antigens, aliquots of freshly detached adherent cells were washed with PBS containing 2% FBS. Cells were

immunolabelled with the following anti-human antibodies: CD49a-PE, CD80-PE, CD45-PE, CD90-PE, CD105-PE, CD73-PE, HLA-DR-PE, LNGFR-PE, SSEA4-PE (Pharmingen). The cells were analysed on a FACSVantage with the CellQuest software (BD Biosciences).

## 2.4. Osteogenic differentiation

To induce osteogenic differentiation,  $3 \times 10^3$  cells/cm<sup>2</sup> were plated in DMEM supplemented with 10% FBS, 10 mM β-glycerophosphate, 60 µM ascorbic acid and 10<sup>-7</sup> M dexamethasone (Sigma-Aldrich), and cultured for 3 to 4 weeks, replacing the medium every 3–4 days. To demonstrate osteogenic differentiation, the cultures were fixed and stained with Alizarin (Sigma-Aldrich).

## 2.5. Adipogenic differentiation

To induce adipocyte differentiation,  $3 \times 10^3$  cells/cm<sup>2</sup> were cultured in DMEM supplemented with 10% FBS, 60 µM indomethacin, 10<sup>-6</sup> M dexamethasone and 5 µg/ml insulin (Sigma). The cells were cultured, replacing the medium every 3–4 days. After 3 to 4 weeks of culture, cells contained neutral lipids in fat vacuoles; they were fixed in 10% formalin and stained with fresh Oil Red O solution (Sigma-Aldrich).

## 2.6. Hepatogenic differentiation

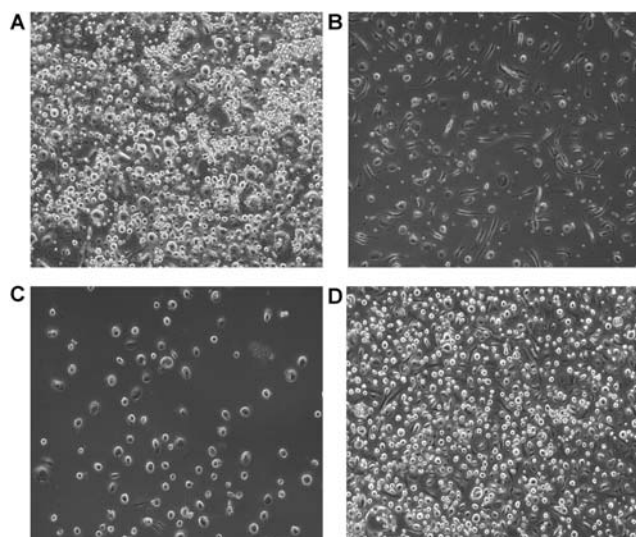
Hepatic differentiation was achieved by sequential addition of exogenous factors. Briefly, MSC from passages 2 to 3 at 80% confluence were precultured for 2 days at  $3 \times 10^3$  cells/cm<sup>2</sup> in DMEM supplemented with 10% FBS. At day 0, cells were cultured in IMDM (Iscove's modified Dulbecco's medium) +10% FBS supplemented with EGF (epidermal growth factor) 20 ng/ml, bFGF (basic fibroblast growth factor) 20 ng/ml (eBioshop) (conditioning step). From day 2 to day 12, MSC were cultured in IMDM+10% FBS supplemented with HGF (hepatocyte growth factor) 20 ng/ml, bFGF 20 ng/ml (eBioshop), nicotinamide 0.61g/l and ITS premix 1% (Sigma-Aldrich) (step 1 of differentiation). From day 12 to 22, the medium was replaced by IMDM+10% FBS, oncostatin M 20 ng/ml, ITS premix 1% and dexamethasone 1 µM (step 2 of differentiation and maturation). The medium was changed twice a week. Glycogen storage was assessed by staining with the Schiff reagent.

Human hepatocytes were cultured in Williams E media (Invitrogen) supplemented with dexamethasone, EGF and insulin.

# 3. Results

## 3.1. Culture of total MNC from UCB

As a first approach, MNC were isolated from UCB units of more than 40 ml processed within 8 to 10 h. Cells were plated at  $(1-3) \times 10^8$  cells in four different culture media, i.e. MSCGM<sup>®</sup> ( $n=10$ ), DMEM+5% platelet lysate ( $n=10$ ), DMEM+10% human UCB serum ( $n=6$ ) and DMEM+10% irradiated FBS ( $n=10$ ). As



**Figure 1 Morphological analysis of UCB-derived adherent cells**  
Morphology of UCB-derived adherent cells after 2 weeks of MNC culture in (A) MSCGM<sup>®</sup> ( $n=10$ ); (B) DMEM+5% platelet lysate ( $n=8$ ); (C) DMEM+10% human UCB serum ( $n=6$ ); (D) DMEM+10% irradiated FBS ( $n=10$ ). Phase contrast magnification  $\times 300$ .

shown in Figure 1 and Table 1, none of the UCB samples tested showed fibroblast progenitors. In contrast, adherent cells in all UCB cultures consisted of single cells distributed throughout the well. Generally, after a week of culture, there was a heterogeneous mix with a predominance of two forms of cells: macrophage-like cells and spindle-shaped cells (Figure 2). When prolonging the culture, the macrophage-like cells tended to dominate, but their proliferative potential remained limited. Contrary to the

spindle-shaped cells, the macrophage-like cells were not detachable by trypsin. This characteristic allowed us to separate and analyse the two types of cells. As shown in Figure 2, the macrophage-like cells were 44% CD14<sup>+</sup>, 86% CD11c<sup>+</sup> and 72% CD11b<sup>+</sup>, whereas the spindle-shaped cells were negative for all MSC markers (CD73, CD90 and CD105).

### 3.2. Culture of enriched or depleted MNC from UCB

In a second series of experiments, we tried to establish MSC cultures from UCB after enrichment in CD133<sup>+</sup> or LNGFR<sup>+</sup> cells or after depletion of CD3<sup>+</sup>, CD19<sup>+</sup>, CD14<sup>+</sup> and CD38<sup>+</sup> cells before the culture. As shown in Figure 3, none of these isolation methods allowed the establishment of MSC cultures. Instead there were no adherent cells after 1 week of culture in any of the media used (MSCGM<sup>®</sup>, DMEM+5% platelet extract, DMEM+10% human UCB serum or DMEM+10% irradiated FBS). The same result was obtained after enrichment for LNGFR<sup>+</sup> cells (data not shown).

### 3.3. Culture and characterization of UCM and BM-derived MSC

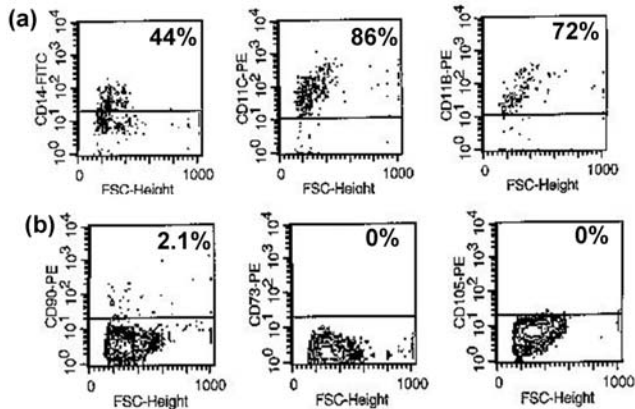
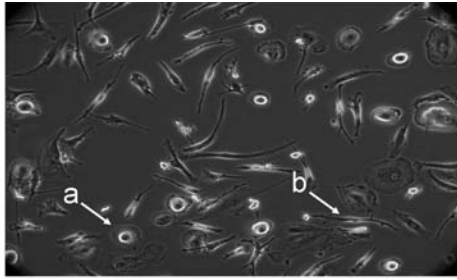
We processed UCM samples and isolated cells that were plated in culture in the presence of DMEM supplemented with 5% platelet lysate. MSC expanded from all the UCM samples processed (8:8). Fibroblast-like colonies started to appear within the first week, reaching confluence after 2 to 3 weeks (Figure 4). The morphology of MSC derived from the UCM was identical to that of MSC isolated from BM. After passaging with trypsin, the fibroblastoid population of cells continued to proliferate even after numerous passages, with growth kinetics being significantly higher for UCM

**Table 1 Overview of MSC isolation techniques and yield in the literature compared with the present study**

Sample selection criteria: 1, collection to isolation <15 h; 2, sample volume >33 ml. Culture medium: 1, DMEM+5% platelet lysate; 2, DMEM+10% FBS; 3, DMEM+10% human UCB serum; 4, MSCGM<sup>®</sup>.

Method	Sample selection criteria	Sample processing	Culture media	MSC yield	References
Plastic adherence of total MNC	Criteria (1) and (2)	Ficoll centrifugation, total MNC > $1 \times 10^8$ cells	Media (1), (2), (3) and (4)	1/15	The present study
	–	Ficoll centrifugation, total MNC > $1 \times 10^8$ cells	Media (2)*	0/14	Gutierrez-Rodriguez et al., 2000
	–	Ficoll centrifugation, total MNC > $1 \times 10^8$ cells	Media (2)	0/58	Mareschi et al., 2001
	–	Ficoll centrifugation, total MNC > $1 \times 10^8$ cells	Media (2)	0/10	Wexler et al., 2003
	Criteria (1) and (2)	Ficoll centrifugation, total MNC > $1 \times 10^8$ cells	Media (4)	17/59	Bieback et al., 2004
Culture of selected CD133 <sup>+</sup> cells	Criteria (1) and (2)	Positive selection of CD133 <sup>+</sup> cells from MNC	Media (1), (2), (3) and (4)	0/3	The present study
	Criteria (1) and (2)	Positive selection of CD133 <sup>+</sup> cells from MNC	$\alpha$ -MEM + 10% FBS	2/8	Tondreau et al., 2005a
Culture of selected LNGFR <sup>+</sup> cells	Criteria (1) and (2)	Positive selection of LNGFR <sup>+</sup> cells from MNC	Media (1), (2), (3) and (4)	0/3	The present study
	–	Positive selection of LNGFR <sup>+</sup> cells from MNC	Media (1) and DMEM+10% pooled allogeneic human serum	100%	Poloni et al., 2009†
Culture of CD3 <sup>+</sup> CD14 <sup>+</sup> CD19 <sup>+</sup> CD38 <sup>+</sup> depleted MNC	Criteria (1) and (2)	Depletion of CD3 <sup>+</sup> CD14 <sup>+</sup> CD19 <sup>+</sup> CD38 <sup>+</sup> cells	Media (1), (2), (3) and (4)	0/4	The present study
	–	Depletion of CD3 <sup>+</sup> CD14 <sup>+</sup> CD19 <sup>+</sup> CD38 <sup>+</sup> cells	$\alpha$ -MEM + 20% FBS	3/10	Barrachini et al., 2009

\* Media (2)+0.2 mM inositol, 20  $\mu$ M folic acid,  $10^{-4}$  M 2-mercaptoethanol and  $10^{-6}$  M hydrocortisone.  
† Experiments on bone marrow samples.



**Figure 2** Appearance under inverted microscopy of a representative culture of MNCs from UCB

Arrows indicate the two types of cells: (a) trypsin-und detachable cells: macrophage-like cells; (b) trypsin-detachable cells: spindle-shaped cells. Phase contrast magnification  $\times 400$ . Flow cytometry analysis was performed on the two types of cells shown in (a) and (b).

than for BM (Figure 4E). Indeed, the cumulative population doubling was higher for UCM compared with BM ( $21.2 \pm 4.5$  compared with  $13.9 \pm 1.2$ ,  $P=0.04$ ) and the doubling time lower for UCM in comparison with BM ( $1.3 \pm 1.1$  compared with  $4.5 \pm 1.9$  days,  $P=0.03$ ).

MSCs cultured from UCM and BM were analysed for the expression of cell surface markers. UCM as well as BM MSC were negative for the haematopoietic antigen CD45 (Figure 5). UCM- and BM-derived MSC expressed the surface proteins CD105, CD73 and CD90. However, MSC from BM were positive for SSEA4 and LNGFR ( $21.2 \pm 15.5$  and  $19.9 \pm 16.6$ , respectively), whereas MSC from UCM were negative for these markers.

UCM MSCs were also tested for their ability to differentiate into adipocytes, osteocytes and hepatocytes. Culture of MSC from passages 3 to 4 with adipogenic, osteogenic and hepatogenic media (Figure 6) revealed a potential to differentiate into osteocytes, adipocytes and hepatocytes, with no difference between UCM and BM. Thus, after switching MSC from the regular culture medium into an osteogenic medium, cells with an osteoblast-like phenotype appeared, as revealed by Alizarin Red staining and by the deposition of a mineralized matrix. Moreover, when MSC were exposed to an adipogenic medium, cells differentiated into adipocytes that displayed a perinuclear accumulation of lipid vacuoles as detected after Oil Red O staining. Cell culture in a hepatogenic medium led to the characteristic polyhydric form of hepatocytes with a capacity of glycogen storage as shown by staining with the Schiff reagent (Figure 6). These hepatocytes

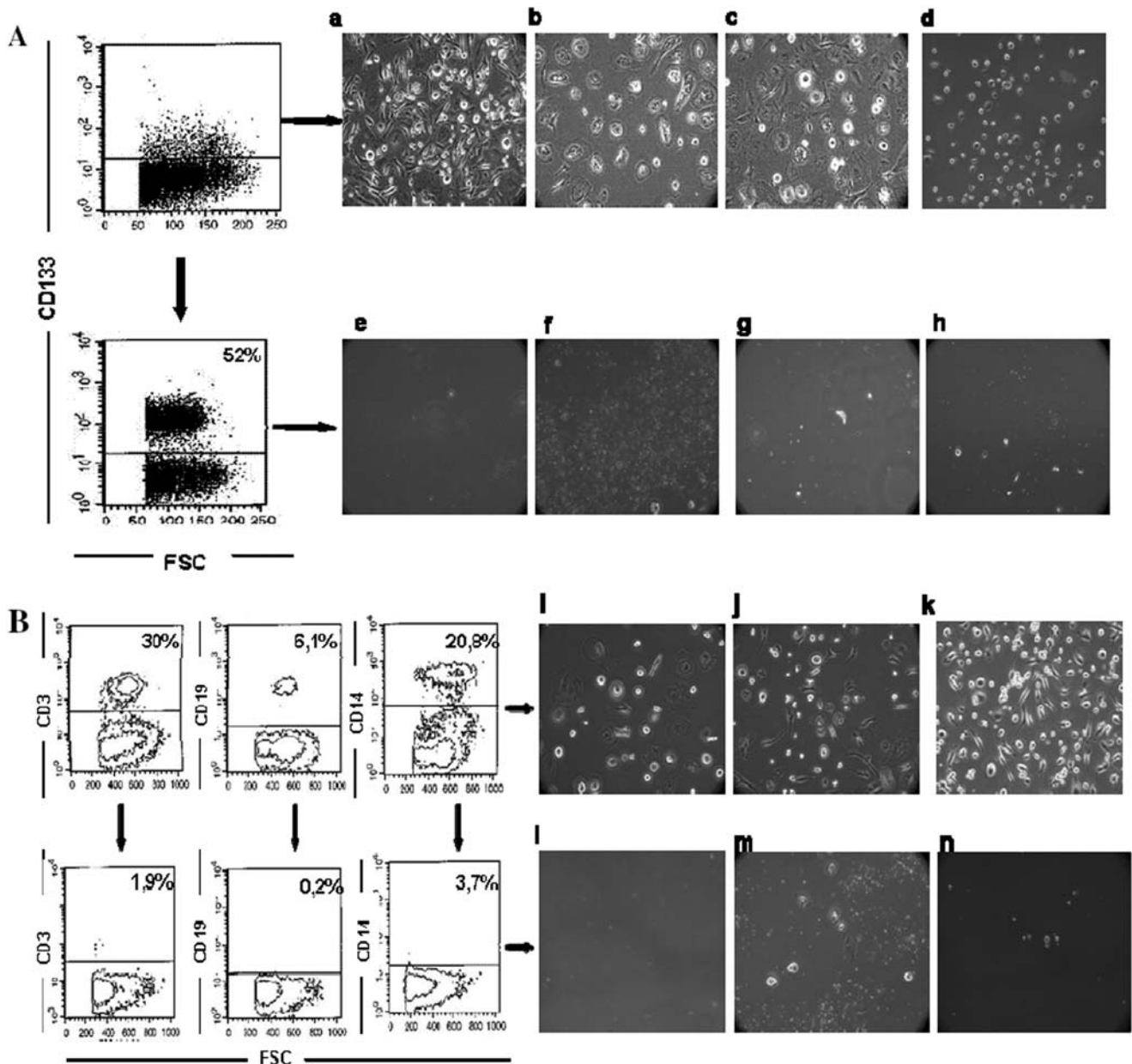
were also positive for albumin (data not shown). The above features were not observed in MSC grown in regular culture medium.

## 4. Discussion

Owing to their capacity to differentiate into different cell types (adipocytes, osteocytes and chondrocytes) (Pittenger et al., 1999; Sekiya et al., 2002), MSC have aroused a great interest in cell and gene therapy. Despite the invasive procedure related to MSC isolation from the bone marrow, it is still considered as the main source of MSC with a frequency of 0.001% to 0.01% depending on the age of the donors (Rao and Mattson, 2001; Van and Liang, 2003). MSC have been shown to reside within numerous fetal organs such as the liver and kidney and to circulate in the blood of preterm fetuses (Campagnoli et al., 2001; Meida-Porada et al., 2002). Given the ease of collecting and storing it, the use of UCB as a source of MSCs has been attempted in many studies. However, the possibility of obtaining MSCs from this source is still controversial.

Here we propose to further settle this question by comparing the methods used in the literature to isolate MSC from UCB. Our results show clearly that, whereas it was possible to isolate MSC from 100% of the BM and UCM samples, only one of the 15 UCB units gave rise to MSC cultures using various parameters of isolation and culture (Table 1). Culture of total MNC led to adherent cells with a phenotype characteristic of monocytes and dendritic cells, in accordance with previous studies that proposed UCB as a source for dendritic cells isolation (Gutierrez-Rodriguez et al., 2000). Enrichment by CD133<sup>+</sup> or LNGFR<sup>+</sup> antibodies simply led to the depletion of all adherent cells, giving rise to empty flasks. The same result was obtained after depletion of CD3<sup>+</sup>, CD19<sup>+</sup>, CD14<sup>+</sup> and CD38<sup>+</sup> cells. CD133 or LNGFR selection have been considered as possible strategies for MSC isolation (Tondreau et al., 2005; Lin et al., 2008; Poloni et al., 2009). In the case of CD133, all results are based on the use of two monoclonal antibodies (CD133/1 or /2) recognizing poorly characterized glycosylated epitopes of cell surface CD133. However, not all cells positive for one antibody will be necessarily positive for the other and one of them displays a cross-reactivity with the keratin-18 protein (Potgens et al., 2002; Bidlingmaier et al., 2008). This may be a possible explanation for the contradictory results obtained after CD133<sup>+</sup> cell selection using these commercial antibodies. Our study showed LNGFR expression on MSC from BM. The successful use of this marker for MSC isolation has been reported for BM (Poloni et al., 2009). We failed to establish MSC cultures after LNGFR enrichment of UCB samples. This may be explained by the absence of such marker on UCB MSC contrarily to BM MSC. CD3<sup>+</sup>/CD19<sup>+</sup>/CD14<sup>+</sup>/CD38<sup>+</sup> depletion is normally performed to limit the possibility that accessory cells, such as macrophages or lymphocytes, could inhibit the proliferation of MSC. However, other cell types could be involved in this inhibition.

The high variability observed in the literature for MSC isolation from UCB could also be related to the mother (mother's age, ethnicity, alcohol and cigarette consumption, haemoglobin levels)

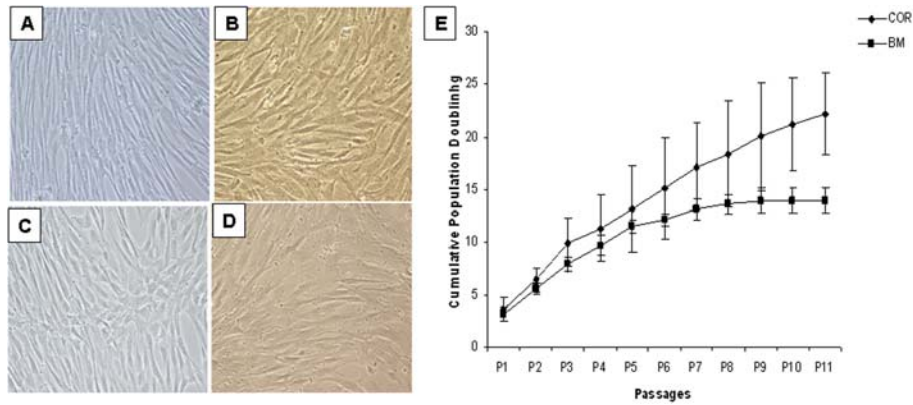


**Figure 3** Morphology of UCB-derived adherent cells after culture of (A) CD133<sup>+</sup>-enriched MNC or (B) CD3<sup>+</sup>/CD19<sup>+</sup>/CD14<sup>+</sup>/CD38<sup>+</sup>-depleted MNC  
 A) MNCs from UCB were enriched for CD133<sup>+</sup> cells ( $n=4$ ) and were cultured in (e) DMEM+10% FBS, (f) DMEM+5% platelet lysate, (g) MSCGM<sup>®</sup>, (h) DMEM+10% UCB serum. Cultures of unpurified MNC in the same media are shown in (a), (b), (c) and (d), respectively. Flow cytometry analysis was performed on cells before and after enrichment. (B) Mononuclear cells from UCB were depleted of CD3<sup>+</sup>, CD19<sup>+</sup>, CD14<sup>+</sup>, CD38<sup>+</sup> cells ( $n=3$ ) and were cultured in (l) MSCGM<sup>®</sup>, (m) DMEM+5% platelet lysate, (n) DMEM+10% FBS. Cultures of unpurified mononuclear cells in the same media are shown in (i), (j) and (k), respectively. Flow cytometry analysis was performed on cells before and after depletion.

or other pregnancy factors (gestational age, Caesarean or natural delivery, placental size, and cord length...). However, this could not be analysed in our study because of the limited number of UCB units tested and the very strict exclusion criteria of our UCB bank. It cannot be excluded that, even if we were unable to isolate MSC among the  $3.1 \pm 1.7 \times 10^8$  MNC from 30–40 ml UCB units, some larger units may provide low numbers of MSC.

It has been reported that MSC could be isolated from preterm UCB (Erices et al., 2000), placenta (Miao et al., 2006), amniotic

membrane (Yu et al., 2009) and UCM (Wang et al., 2004a; Fu et al., 2006; Seshareddy et al., 2008; Zhang et al., 2009), the latter appearing as the most interesting for clinical use. We confirmed this finding by showing that MSC could be cultured from 100% of tested samples. MSC from UCM presented the same aspect as MSC from BM but with a significantly higher proliferative potential when cultured in DMEM supplemented with 5% human platelet lysate reported to contain multiple growth factors (Schwartz-Arad et al., 2007). The use of this medium could be an excellent



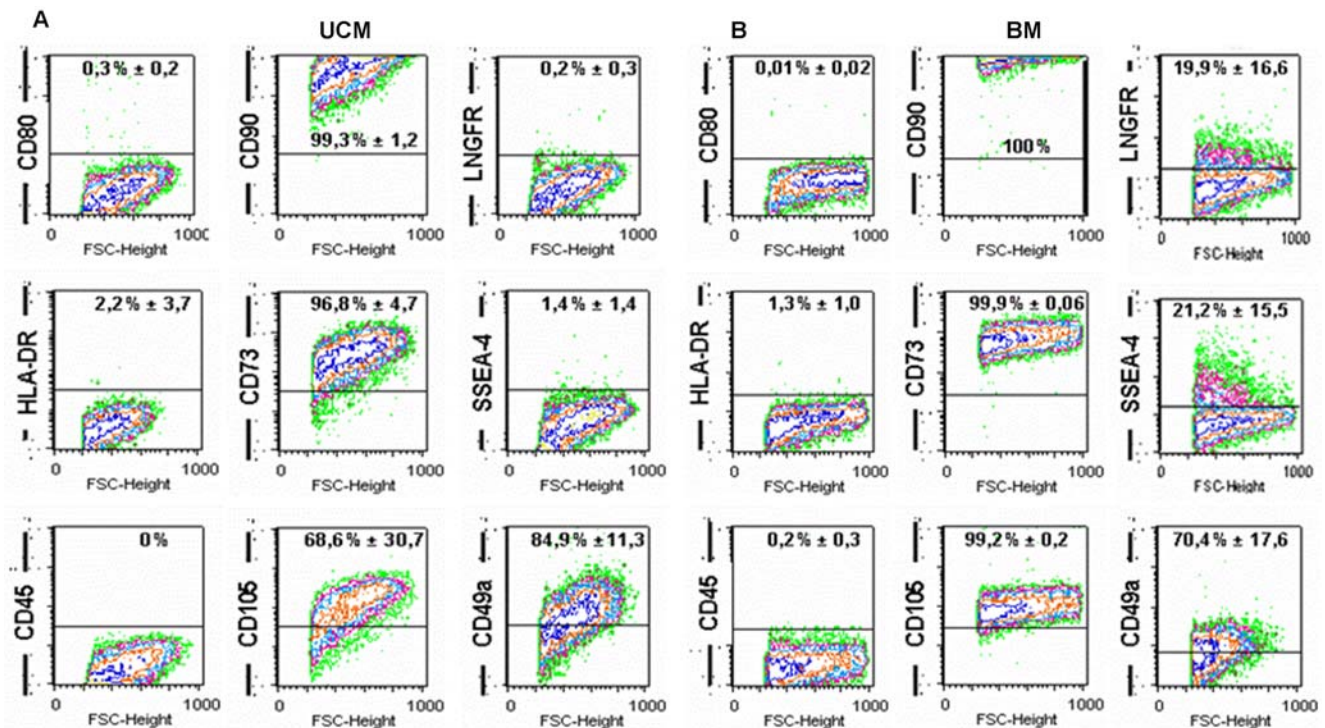
**Figure 4 Morphology and proliferation potential of MSCs derived from UCB**  
Morphology of MSC derived from UCB at (A) passage 1 (B) passage 7, or from BM at (C) passage 1 and (D) passage 4. (E) Mean values of the cumulative population doublings, determined at each passage of MSC from UCM ( $n=8$ ) and BM ( $n=10$ ).

alternative to the use of the animal FBS, being less suitable for infusion into human beings.

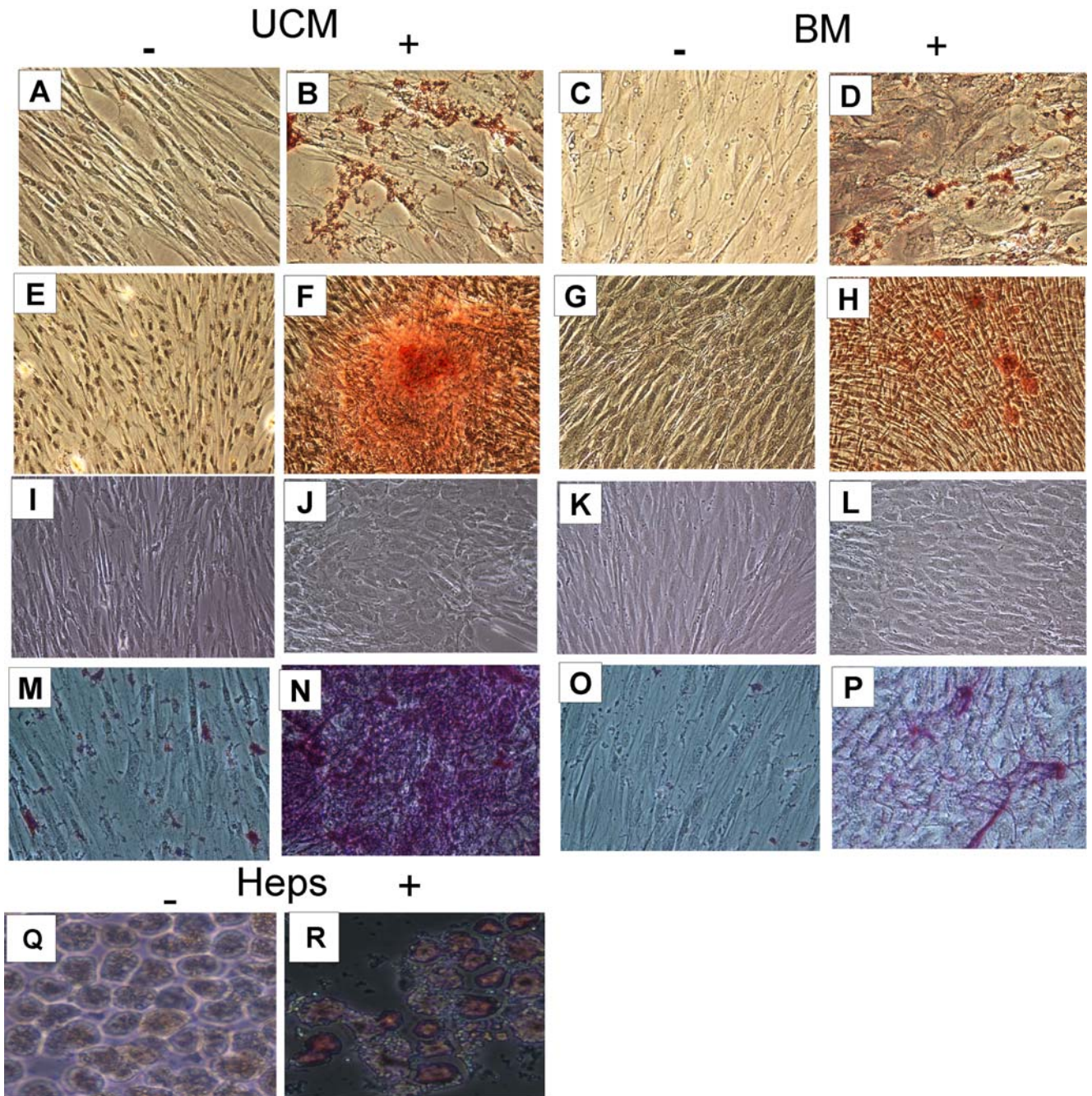
We also demonstrated, as previously described (Wang et al., 2004; Campard et al., 2008), the ability of MSC from UCM to differentiate into adipocytes, osteocytes and hepatocytes.

There was, however, a difference in the pattern of expression of some phenotypic markers. In fact, whereas UCM MSC were totally negative for SSEA-4 and LNGFR antigens, BM MSC presented a fraction of SSEA-4<sup>+</sup> and LNGFR<sup>+</sup> cells. This expression was not restricted to a particular fraction of BM

MSC, given that sorted negative cells were able to re-acquire the expression after 1 week of culture (data not shown). SSEA-4 (an early embryonic glycolipidic antigen commonly used as a marker for undifferentiated pluripotent human embryonic stem cells) and LNGFR (CD271) (involved in the development, survival and differentiation of neural cells) have been proposed to identify the adult mesenchymal stem cell population (Quirici et al., 2002; Gang et al., 2007). The absence of these two markers on UCM MSC may suggest a less primitive stage in comparison with BM MSC.



**Figure 5 Immunophenotype of MSC from UCM and BM**  
Cultured cells from UCM (A) and BM (B) harvested at passage 3 were labelled with antibodies against indicated antigens and analysed by flow cytometry. Values represent the mean percentage ± S.D. of all assessed cells positively stained by the respective antibodies.



**Figure 6** Differentiation capacity of MSC from UCM and BM

Cultured cells from UCM and BM harvests were exposed *in vitro* to differentiation media to induce adipogenic, osteogenic and hepatogenic differentiation, respectively. UCM MSC (B) and BM MSC (D) were shown to differentiate appropriately into the adipogenic lineage by Oil Red O staining. Controls are shown in (A) and (C), respectively. Osteogenic differentiation is shown for UCM MSC (F) and BM MSC (H) by illustrating calcium mineralization using Alizarin Red staining. Controls are shown in (E) and (G), respectively. Hepatogenic differentiation is shown by the appearance of the characteristic polyhedral shape of hepatocytes for UCM MSC (J) and BM MSC (L), and by the demonstration of glycogen storage by staining with the Schiff reagent UCM MSC (N) and BM-MSC (P), respectively. Controls are shown in (I) and (M) for UCM MSC and (K) and (O) for BM MSC, respectively. Control unstained and Schiff reagent-stained human hepatocytes are shown in (Q) and (R), respectively.

## 5. Conclusion

According to the critical parameters of sample selection described in the literature, and using different culture media proposed to enhance the growth of MSC, in parallel with the use of different methods of cell isolation, we were not able to establish MSC cultures from more than one out of 15 UCB samples. Given the high frequency of MSC in UCM, we hypothesize that there may be MSC contamination while collecting cord blood. This may explain the rare described cases where MSC isolation from UCB has been possible. However, it could not be ascertained whether the collection method may have caused the disappearance of circulating MSC from the cord blood MNC compartment in favour of the endothelial/subendothelial layer of the UCM. Anyway, UCB can be excluded as a reliable source of MSC in favour of the richer and more reproducible source that is the UCM.

### Author contribution

Mustapha Zeddou conceived the study, carried out the experiments and drafted the manuscript. Alexandra Briquet participated in performing experiments. Biserka Relic participated in conceiving the study. Claire Josse participated in performing experiments. Michel Malaise helped in conceiving the study. André Gothot helped in conceiving the study. Chantal Lechanteur conceived the study and helped to carry out the experiments. Yves Beguin conceived the study, and drafted the manuscript. All the authors read and approved the final manuscript.

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