Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, cord blood and matrix

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Background
Mesenchymal stem cells (MSCs) represent an archetype of adult multipotent somatic stem cells and a promising tool for application in regenerative medicine. However, researchers do not concur on the best source for expansion and clinical differentiation.

Aim
The current study was performed to evaluate and compare adipose tissue (AT), Wharton's jelly (WJ), and umbilical cord blood (CB) with bone marrow (BM)-MSCs in terms of their morphology and their expansion potentiality using the minimum requirements for growth.

Participants and methods
Mechanical–enzymatic protocol was used for isolation of AT-MSCs and WJ-MSCs, whereas density-gradient separation method and automated cell separator were applied for isolation of BM-MSCs and CB-MSCs, respectively. The isolated cells were cultured in RPMI-1640 media containing fetal bovine serum for 6 days. Then, their phenotypic characterization (CD34, CD133, CD90, and CD105) and their viability (7-aminoactinomycin D) was detected by flow cytometry.

Results
Our results showed that MSCs from the four different sources have similar morphology, the highest percentage of CD34, CD133, and CD90 was found in CB samples, whereas the lowest was for BM samples. While the highest percentage of CD105 was expressed in AT samples and the lowest was also present in BM samples. Finally, the BM-MSCs showed the least liability to be damaged following the handling procedure for separation and culturing, whereas WJ-MSCs were the most injured ones. This may be correlated to the mechanical–enzymatic protocol used for cells' isolation.

Conclusion
Although BM and AT specimen collection involves painful procedures, being autologous makes them safe and feasible candidates for therapeutic future applications.

Keywords:
adipose tissue, cell culture, cord blood, mesenchymal stem cells, Wharton's jelly

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Introduction

Mesenchymal stem cells (MSCs) are a group of undifferentiated, ubiquitous, multipotent, nonhematopoietic somatic cells having the property of attaching to plastic and forming colonies that are capable of self-renewal. Previously, they were recruited from bone marrow (BM), but presently, they can be isolated from almost every tissue of the body [1].

MSCs have generated great interest in clinical-therapy applications, for the reason that they can be isolated from different sources such as BM, blood, trabecular bone, adipose tissue (AT), umbilical cord blood (UCB) and tissue (CB and CT), dermis, synovium, skeletal muscle, and pericytes [2].

The ideal stem cell population, proposed for regenerative medicine, should be accessible in abundant numbers, harvestable by a relatively noninvasive procedure, able to differentiate into a variety of cell lineages, easy to transplant to an autologous or allogeneic host, and able to be manufactured in accordance with the currently accepted guidelines set by the FDA [3].

However, researchers do not concur on the best source of stem cells for expansion and clinical differentiation. Hence, mesenchymal and tissue stem cell committee of the International Society for Cellular Therapy has projected minimal criteria to describe human MSCs: MSCs must be plastic adherent, express CD105, CD73, and CD90 and lack expression of CD45, CD34, and CD14 or CD19 and HLA-DR surface molecules, at the same time capable of differentiation into osteogenic, adipogenic, and chondrogenic lineages [4].

In 2012, Wakao *et al.*[5] clarified the triploblastic differentiation potential of MSCs, while MSCs

originate from the mesoderm, they are capable of crossing boundaries from mesodermal to ectodermal and endodermal lineages for both *in vivo* and *in vitro* trials.

Considering the International Society for Cellular Therapy and Wakoa-team suggestions, many researchers had used MSCs isolated from UBC-MSCs, Wharton's jelly (WJ)-MSCs, BM-MSCs, and AT-MSCs to demonstrate their similarities and differences regarding the morphology, phenotype, and differentiation potential [6].

Various isolation and characterization protocols have been proposed for cultivation of MSCs, many of which are expensive and time consuming [7].

Additionally, many studies revealed that variations in conditions have a significant impact on the expansion potential and phenotypic characterization of the generated populations, albeit the initial cell source could be phenotypically identical [8].

Therefore, this pilot study was intended to describe and compare the morphological and immunophenotypic characteristics of different sources of MSCs after culturing in simplified media containing the minimal requirements for cell growth.

Patients and methods

A total of 24 samples from BM, AT, CB, and cord tissue (six from each) were obtained from 24 healthy donors recruited from Dar El Salam and El Monira Hospitals, Cairo, Egypt, during the period from September 2016 to March 2017. Data confidentiality was conserved according to the Revised Helsinki Declaration of Bioethics (2008). Informed consent was obtained from adult donors and mothers of the newborn babies.

Sample collection and mesenchymal stem cells isolation from bone marrow

BM aspirates (5–6 ml) were collected in preservative-free heparinized tubes from the posterior iliac crest of six volunteers.

BM-MSCs were isolated by Ficoll density separation method by layering the diluted sample on top of Ficoll and then centrifugation was done at 500 rpm for 30 min to separate the buffy-coat layer containing the MSCs. The mononuclear cell (MNC) layer was carefully transferred to a new Falcon tube, washed in phosphate-buffer saline (PBS), and then centrifuged at 1500 rpm for 10 min to form a pellet.

Sample collection and mesenchymal stem cells isolation from adipose tissue

AT samples (3 g) were obtained from six healthy donors undergoing elective abdominal surgeries. Samples were preserved in Falcon tubes containing PBS supplemented with antibiotic/antimycotic mixture and then transferred immediately to the laboratory in a sterile icebox.

AT-MSCs were isolated using mechanical and enzymatic protocol, where the AT sample was cut into small pieces with sterilized scissors in a petri dish containing 2 ml of prewarmed trypsin-EDTA, then placed in a Falcon tube, and centrifuged at 1500 rpm for 10 min to form the MNC pellet.

Sample collection and mesenchymal stem cell isolation from cord blood

UCB samples were obtained from full-term deliveries from the umbilical vein with the placenta in utero after fetal delivery with C sections. After cleaning the umbilical cord with betadine (bovidine iodine) and alcohol, about 10–15 ml of blood was collected under complete aseptic conditions on preservative-free heparin as anticoagulant. The CB was directly placed in a collecting blood bag.

CB-MSCs were isolated by automated cell separator of AXP system where samples were placed in the processing set and then centrifuged twice to separate the MSCs in the freezing bag.

Sample collection and mesenchymal stem cell isolation from cord tissues

Fresh human umbilical cord segments (3 cm) were obtained after full-term cesarean-section deliveries, preserved in PBS solution in Falcon tubes, and transported to the laboratory facilities in a sterile ice box.

CT-MSCs were isolated by mechanical–enzymatic protocol starting with cutting the umbilical cord segment longitudinally to expose and separate the matrix with addition of prewarmed trypsin-EDTA and then centrifuged at 1500 rpm for 10 min to form the pellet containing the MSCs.

Mesenchymal stem cell culture

The supernatant of all processed samples was discarded, while the pellet (1 ml) was placed in a culture flask with 9 ml of the culture medium. The culture-medium constituents are RPMI 1640 (Bio-Wittaker; Lonza, Miltenyi Biotec, Beckman Coulter, IBM also Bio-Wittaker, Lonza. Address: 8830 Biggs Ford Road, Walkers Ville, MD21793. Maryland, USA., Cat.N:

BE12-115F), fetal bovine serum (PAA. Cat.N: A11-151), and penicillin–streptomycin–amphotercin B mixture (Bio-Wittaker; Lonza, Cat.N: BE17-602F) in the ratio 5 : 1 : 0.05, respectively. Then, the cells were examined under the inverted microscope. Next, the cells were incubated at 37°C in 5% humidified CO_2 for 6 days. Medium change was done on the third day.

Mesenchymal stem cell harvest

Upon development of colonies, flasks were washed with PBS and cells were detached with prewarmed trypsin-EDTA for 10–15 min at 37°C, then the flasks were rocked. Two milliliters of the medium were added to each flask to neutralize excess trypsin. Flasks were examined under an inverted microscope to observe the detachment of cells. Cell suspension was transferred to a 15-ml Falcon tube and centrifuged at 1500 rpm for 15 min forming cellular pellets. The supernatant was discarded and then 2 ml of fresh RPMI was added to the pellet. MSC markers [CD34 (Miltenyi Biotec, USA) (Cat. N: 130-081-001); CD90 (R&D) (Cat.N: FAB2067P); CD105 (Miltenyi Biotec, USA) (Cat. N: 130-098-778); CD133 (Miltenyi Biotec) (Lot number: 130-080-901)] and 7-aminoactinomycin D (7-AAD) (Miltenyi Biotec) (Cat. N: 130-111-568) were estimated by flow cytometer (Beckman Coulter).

Statistical methodology

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) computer software (version 22), IBM Software, USA. All data were expressed as means ± SEM. One-way analysis of variance test was used to elucidate significance between group means, followed by Tukey's post-hoc test for pairwise comparisons. Correlation was done by Spearman's correlation to test the association between different parameters. It was considered statistically significant at *P* value less than 0.05.

Results

The highest percentage of CD34, CD133, and CD90 was found in CB-MSCs, whereas the lowest was for BM-MSCs. There was a statistically significant increase in the mean value of CD34 and CD133 in cultured CB-MSCs when compared with AT-MSCs, BM-MSCs, and CT-MSCs.

Cultured CB-MSCs showed the highest level of CD90 with a statistically significant difference compared with BM-MSCs and CT-MSCs. Albeit CD90 percentage of CB-MSCs was higher than AT-MSCs, this increase was statistically insignificant.

On the contrary, the highest percentage of CD105 was for AT-MSCs and the lowest was also for BM-MSCs. Additionally, this increase was statistically significant when compared with its level on the three other tissues' derived MSCs.

All data were illustrated in Table 1 and Fig. 1.

Viability of cells was also evaluated by measuring 7-AAD percentage. BM-MSCs showed the highest values, whereas CT-MSCs expressed the lowest ones. This was regarded to the handling procedure for separation and isolation (mechanical–enzymatic protocol). BM-MSCs' viability percentage was statistically significant when compared with AT-MSCs and CT-MSCs. Meanwhile, the difference between BM-MSCs and CB-MSCs was statistically insignificant.

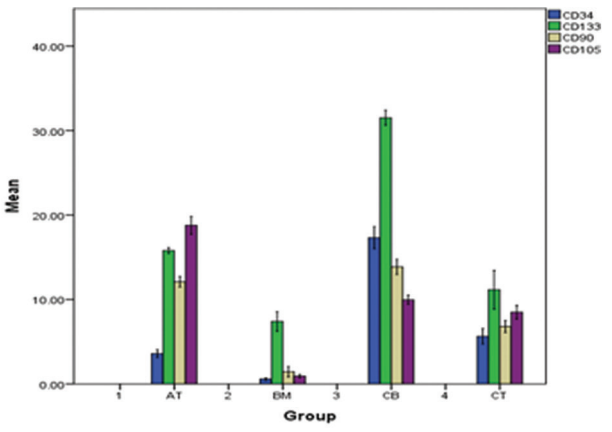
While the correlations between different surface markers on the different MSC sources were statistically insignificant, AT showed a statistical significant negative correlation between CD34 and CD105 (Table 2).

Discussion

MSCs represent a promising tool for new clinical concepts concerning cellular therapy, as a result of their facilitated obtainment and immunologic privilege [9].

The most popular source of MSCs is BM as it is relatively simply collected through BM aspiration. Then, the BM aspirates or MNCs, isolated by a Ficoll gradient, are suspended in medium cultured adherently on plastic dishes [10].

Figure 1



Multiple comparisons of different CD markers from the four sources of MSCs. MSC, mesenchymal stem cell.

Table 1 Comparison between the percentage of CD34, CD133, CD90, CD105, and 7-aminoactinomycin D in the different mesenchymal stem cells sources

Groups	CD34	CD133	CD90	CD105	7-AAD (average)
AT	3.59±0.47	15.80±0.34	12.10±0.64	18.77±1.06	95.60±0.84
BM	0.57±0.14	7.39±1.16[a]	1.44±0.62[a]	0.92±0.21[a]	99.28±0.10[a]
CB	17.32±1.32[ab]	31.52±0.89[ab]	13.87±0.92[b]	9.97±0.54[ab]	97.53±0.29
CT	5.63±0.92[bc]	11.14±2.29[c]	6.80±0.72[abc]	8.49±0.82[ab]	93.51±0.46[abc]

Each value represents a mean of 6 values±SEM. 7-AAD, 7-aminoactinomycin D; AT, adipose tissue; BM, bone marrow; CB, cord blood; CT, cord tissue. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey's post-hoc analysis. [a]Significantly different from AT value at $P<0.05$. [b]Significantly different from BM value at $P<0.05$. [c]Significantly different from CB value at $P<0.05$.

Table 2 Correlations between CD34, CD133, CD90, CD105, and 7-aminoactinomycin D in adipose tissue-mesenchymal stem cells

	CD34	CD133	CD90	CD105	7AAD
CD34					
Pearson correlation	1	−0.632	−0.361	−0.903	0.248
Sig. (2-tailed)		0.178	0.482	0.014	0.636
n	6	6	6	6	6
CD133					
Pearson correlation	−0.632	1	0.427	0.678	−0.225
Sig. (2-tailed)	0.178		0.399	0.139	0.669
n	6	6	6	6	6
CD90					
Pearson correlation	−0.361	0.427	1	0.618	−0.504
Sig. (2-tailed)	0.482	0.399		0.191	0.308
n	6	6	6	6	6
CD105					
Pearson correlation	−0.903	0.678	0.618	1	−0.444
Sig. (2-tailed)	0.014	0.139	0.191		0.377
n	6	6	6	6	6
7-AAD					
Pearson correlation	0.248	−0.225	−0.504	−0.444	1
Sig. (2-tailed)	0.636	0.669	0.308	0.377	
n	6	6	6	6	6

7AAD, 7-aminoactinomycin D.

Later on, Zuk *et al.*[11] isolated AT-MSCs from liposuction aspirates using collagenase. Then, the isolated cells were cultured in plastic flasks.

Meanwhile, CB or WJ-derived UC-MSCs have the great advantage of being totally safe without any harm or invasive procedure to the donor. When collecting MSCs from UCB, cells that adhere to a plastic dish are considered as UC-MSCs. To isolate UC-MSCs from WJ, minced tissues are placed on a plastic dish, and the cells that migrate from the tissues are considered as UC-MSCs [12].

Various isolation and characterization protocols have been proposed for cultivation of MSCs, many of which are expensive and time consuming [7].

Additionally, many studies revealed that variations in conditions have a significant impact on the expansion potential and phenotypic characterization of the generated populations, despite that the initial cell source could be phenotypically identical [8].

Therefore, we performed a study to evaluate and compare AT, WJ, and CB with BM-MSCs in terms of their morphology and their potential to expand under standardized conditions.

In this study protocol, 24 samples from BM, AT, WJ, and UCB-MSCs were expanded in a static, serum-added, liquid culture for 6 days in a 90-ml culture flask. Subsequently, we compared MSCs derived from these sources to determine the impact of the simplest culture conditions on morphology, immune-phenotypic expression, and viability percentage.

The current study showed that the BM, AT, WJ, and UCB-derived cells had similar spindle-shaped, fibroblastic morphology consistent with MSC characterization. This finding is in accordance with that reported by Kern *et al.*[13] and Kellner *et al.* [14].

Regarding CD34, it was recommended to be a negative marker for MSCs [4]. However, many researchers demonstrated that many cell types that are CD34+ when freshly isolated (e.g. HSCs and endothelial cells) lose CD34 expression in culture [15,16].

Moreover, the AT-derived MSCs (AT-SCs) are generally classified as CD34+, despite the well-known phenomenon of losing CD34 expression in culture [17]. That is why CD34 should be reevaluated as a truly negative marker for MSCs [18].

Our study revealed that after 6 days of culture in serum-enriched RPMI medium, the highest expression of CD34+ cells was displayed on cultured CB-MSCs. Furthermore, this increase was statistically significant when compared with the mean value of CD34+ cells derived from AT, BM, and CT.

In contrast to our results, Wagner *et al.*[19] reported that no phenotypic differences were observed by flow cytometry regarding the CD34 MSC-derived cells from BM, AT, and UCB. This result was confirmed in 2015 by the group of Secunda *et al.* [2], who added the WJ-MSCs to the previous tissues, revealing the same results.

As for the stemness marker CD133, our research showed a statistically significant increase in the mean value of CD133+ cultured cells derived from CB-MSCs, when compared with MSCs derived from AT, BM, and CT. In comparison with our result, Secunda study (2015) revealed no statistical difference in MSC CD133 expression derived from BM, AT, UCB, and CT.

Although CD90 was recommended as MSC-positive marker, it did not appear to be a useful marker for *in vivo* MSC detection due to lack of cell-type specificity [20].

Our study revealed that cultured CB-MSCs showed the highest percentage of CD90+ cells with a statistically significant difference compared with the MSCs derived from the BM and CT. Although CD90+ CB-MSCs were higher than that expressed on AT-MSCs, this increase was statistically insignificant.

Many researchers had demonstrated the expression of CD90 and CD105 in MSCs from UCB, BM, AT, and CT [21–23]. Recently, Baghaei *et al.*[8] proved that purified MSCs from BM were positive for CD90 and CD105 surface markers.

Furthermore, we found that the highest level of CD105+ cells was expressed by the second passage from AT-MSCs. Additionally, this increase was statistically significant when compared with its level on the three other tissue-derived MSCs.

These results were in accordance with Varma *et al.*[24] study, who reported that AT-MSCs expressed CD105 at low levels when freshly isolated, but its level has raised upon culture passages.

One of our study goals was identifying the appropriate MSCs that can tolerate laboratory manipulations and culture conditions. We used 7-AAD, a nucleic acid dye, to assess the viability of the second-passage harvest. Although positively stained cells are considered nonviable as 7-AAD has crossed the damaged-cell membrane, 7-AAD viability was expressed as a percent of 7-AAD-negative populations among the cell populations [25].

Comparing the mean value of 7-AAD viability, we found that BM-MSCs showed the least liability to be damaged following the handling procedure for separation and culturing. Moreover, there was a statistically significant difference between BM-MSCs and that derived from AT and CT. Meanwhile, the difference between BM-MSCs and CB-MSCs was statistically insignificant.

In 2015, Secunda *et al.*[2] used propidium iodide staining (flow cytometry) to compare the viability of MSCs from BM, AT, UBC, and WJ. They revealed the same as our finding that viable MSCs were higher with BM-derived MSCs [2].

Moreover, we tried to find correlations between the different surface markers in each tissue of our research. Unfortunately, there were not any correlations, except a negative one between CD34 and CD105 in AT-MSCs.

In conclusion, our results can be summarized as follows: the MSCs from the four different sources have similar morphology (spindle-shaped adhering cells), the highest percentage of CD34, CD133, and CD90 was found in CB samples, whereas the lowest was for BM samples. While the highest percentage of CD105 was expressed in AT samples and the lowest was also present in BM samples.

Regarding viability, the BM-MSCs showed the highest values, whereas CT-MSCs expressed the lowest ones. This may be correlated to the mechanical protocol used for cells' isolation.

Finally, we suggest that BM and AT are the most suitable MSC sources for autologous therapeutic applications, despite the painful procedures to get them.

Recommendations

Further studies are needed to better understand the similarities and/or difference of MSCs from various tissues, the optimum culture media used for their expansion, and the ability to cross-talk with primary cells and local environments. This would lead to the optimization of better and potentially safer cellular therapies for treatment of diseases.

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Conflicts of interest

There are no conflicts of interest.

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