Comparable osteogenic capacity of mesenchymal stem or stromal cells derived from human amnion membrane and bone marrow

Mehran Ghasemzadeh · Ehteramolsadat Hosseini · Mohammadhossein Ahmadi ·
Maedeh Kamalizad · Naser Amirzadeh

Received: 14 August 2017 / Accepted: 2 December 2017 / Published online: 5 January 2018
© Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract So far, substantial attentions have been attracted to the application of mesenchymal stem or stromal cells (MSCs) in different therapeutic approaches. Although human bone marrow is commonly considered as a major source for MSCs, having an invasive collection method, ethical consideration and donor availability create a challenge for scientists, leading them to explore better alternative sources for MSCs. The study presented here aimed to characterize and compare osteogenic capacity of MSCs obtained from the amnion membrane (AM) with those originated from BM. Cells isolated from AMs and BMs were cultured in DMEM-low glucose supplemented with FBS, penicillin and streptomycin. After 24 h of incubation, cells adhered to the plastic surface of the flasks were allowed to proliferate for more days. A

sub-confluent culture of cells was trypsinized and re-cultured. The MSCs were characterized by the expression of specific markers with flow cytometry. The osteogenic differentiation of MSCs was also validated by alkaline phosphatase and alizarian red S staining. Our results showed comparable expression of MSCs specific markers for both MSC sources (AM and BM). We also showed the optimum osteogenic differentiation of MSCs from both sources whereas hAM-MSCs revealed higher proliferation rate. We found no essential immunophenotypic differences between MSCs originated from bone marrow and amnion membrane while their differentiations into osteoblastic lineage were also comparable. This was in addition to the higher proliferation rate observed for hAM-MSCs which suggests hAM as an easily accessible and reliable source of MSCs applicable for bone engineering, regenerative medicine or other therapeutic approaches.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10616-017-0177-1>) contains supplementary material, which is available to authorized users.

Mehran Ghasemzadeh and Ehteramolsadat Hosseini have contributed equally to this work.

M. Ghasemzadeh · E. Hosseini (✉) ·
M. Ahmadi · M. Kamalizad · N. Amirzadeh
Blood Transfusion Research Center, High Institute for
Research and Education in Transfusion Medicine, Iranian
Blood Transfusion Organization Building, Hemmat
Express Way, Next to the Milad Tower,
Tehran 14665-1157, Iran
e-mail: e.hosseini10@yahoo.com.au

Keywords Amnion membrane · Bone marrow ·
Mesenchymal stem or stromal cells · Osteogenic

Introduction

As multipotent progenitors, mesenchymal stem or stromal cells (MSCs) are considered to be a promising source for tissue engineering application and cell therapy.

Whilst MSCs were first identified as a part of bone marrow (BM) stromal scaffolds, so far, these cells have been also isolated from various other tissues including adipose (Zannettino et al. 2008), heart (Hoogduijn et al. 2007), Wharton's jelly (Kuo Ching Chao 2008), dental pulp (Jo et al. 2007), peripheral blood (He et al. 2007), cord blood (Oh et al. 2008), menstrual blood (Du et al. 2016; Ren et al. 2016) and more recently, amnion membrane as well as chorionic villi (Yang et al. 2013). This suggests multipotential capacities of MSCs in tissue regeneration, maintenance and repair. In addition, under the specific condition in vitro, MSCs can differentiate into different mesenchymal tissues including osteoblast, adipocyte, chondrocyte and myoblast (Herzog et al. 2003; Madrigal et al. 2014). MSCs capacity for extensive self-renewal and their multilineage differentiation potential also introduced them as multipotent precursors (Pittenger et al. 1999) in different therapeutic approaches (Barry 2003; Ochsenbein-Kolble et al. 2003; Caplan 2009; Sensebe and Bourin 2009; Honmou et al. 2012; Li et al. 2013; Wang et al. 2016), including tissue engineering and regenerative medicine (Tae et al. 2006; Caplan 2007; Mobasheri et al. 2009), injury and wound healing (Jackson et al. 2012; Nuschke 2014), stem cell transplantation (Bernardo and Locatelli 2016; Zhao and Liu 2016) and cell therapy (Wei et al. 2013; Ding et al. 2015) or engraftment of various organs (Wei et al. 2013).

Bone marrow is considered as a major source for MSCs. Most of the stromal cells presented in the BM are generated by MSCs, which are comprised a part of the hematopoietic stem cell niche (Pontikoglou et al. 2011; Fajardo-Orduna 2015). These cells express a variety of adhesive molecules, receptors and soluble growth factors which are critically involved in hematopoietic cell interactions and support hematopoiesis (Verfaillie 1998; Deans and Moseley 2000; Fujita et al. 2008; Miura 2013). Several studies have shown that expanded mesenchymal stromal cells can effectively support hematopoietic cell proliferation by the expression and release of different important growth factors including IL-12, IL-11, IL-6, IL-8, SCF, M-CSF, IL-14, LIF, IL-1, IL-1 β and Flt-3-ligand (Deans and Moseley 2000). Therefore, due to the MSCs' capacity to support hematopoiesis, these cells have been considered as an efficient stromal layer for the expansion of hematopoietic stem cells or their progenitors (Celebi et al. 2011; Walenda et al. 2011;

Klein et al. 2013; Schmal et al. 2016), which are applicable for therapeutic purposes in leukemia patients.

Morphologically, MSCs are characterized as spindle-shaped fibroblastoid cells with few long and thin protrusions around the cell body which restrains a large and round nucleus with a prominent nucleolus (Ogura et al. 2004). MSCs are typically characterized by the expression of the surface markers CD90, CD105, CD73 in the absence of CD14, CD34, CD45 and HLA-DR (Conrad et al. 2008; Madrigal et al. 2014). Osteogenic capacity of MSCs (their differentiation into osteoblasts) is usually utilized to characterize these stromal cells (Fajardo-Orduna et al. 2016). Osteoblasts are responsible in bone formation and its remodeling process which establish skeletal architecture by the production of extracellular matrix proteins and regulators of matrix mineralization (Neve et al. 2011). Thus, producing alkaline phosphatase and/or osteocalcin in cells can confirm successful differentiation of MSCs into osteoblasts in any experimental setting (Deans and Moseley 2000). MSCs' differentiation into osteoblasts provides appropriate seed cells for bone tissue engineering or therapeutic approaches. In addition, human osteoblasts release the spectrum of hematopoietic growth-promoting cytokines which may play roles in hematopoiesis (Jung et al. 2005; Shiozawa et al. 2008). These are findings that suggest osteoblasts derived from MSCs as a stromal feeder layer to support the expansion of hematopoietic precursors (Huang et al. 2006; Chen et al. 2008; de Barros et al. 2010).

Although BM is considered as a major source of stromal cells, the isolation of MSCs from bone marrow could be hampered by its accessibility which is usually complicated further with ethical considerations. Having an invasive collection method, access to consented healthy volunteers, lower number of MSCs obtained from aged donors as well as the painful procedure of its collection are some important limitations. In addition, MSCs separation from this source is somewhat complicated by the cellular heterogeneity and growth of contaminating hematopoietic precursors (Hung et al. 2002; Ramakrishnan et al. 2013).

To rule out these issues, the isolation of MSCs from more accessible sources could be addressed as suitable alternatives, provided that MSCs originated from these sources show comparable efficacy and quality. Several studies suggested neonatal tissues

including cord blood, Wharton's jelly or placenta as appropriate alternative supplies for MSCs. Although the isolation of MSCs from these sources is technically complicated, since they are routinely discarded post-partum, these sources can be widely applicable for extensive research without being ethically objectionable.

Amnion membrane can be also considered as another source from which the MSCs isolation is technically more achievable. That is a thin layer which is easily peeled from the placenta and has less tissue involvement. Therefore, its digestion process is more cost-effective and much easier than solid tissues like cord blood or placenta. The study presented here investigates whether the expansion of human amnion membrane (hAM)-MSCs and its differentiation capacity to osteoblasts can be comparable to those of hBM-MSCs. If so, hAM-MSCs per se can also be considered as an appropriate source for bone tissue engineering or other therapeutic approaches.

Methods and materials

Chemicals and reagents

Mouse IgG1 Isotype control antibodies (PE and FITC conjugated) were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). Mouse anti-human CD90, CD44, CD45 and CD14 (FITC conjugated) as well as CD29, CD73, CD105, CD166 and CD34 (PE conjugated) monoclonal antibodies were purchased from BD Bioscience (Franklin Lakes, NJ, USA). Low glucose Dulbecco's modified Eagle medium (DMEM), Tyrosin-EDTA, collagen, collagenase type IV, SIGMA FAST BCIP/NBT substrate and trypan blue were from Sigma (ST. Louis, MO, USA). We also purchased fetal bovine serum (FBS), penicillin and streptomycin antibiotics from Thermo Fisher (Waltham, MA, USA), osteogenic differentiation medium (NH OsteoDiff medium from Miltenyi Biotec (Auburn, CA, USA), alizarian red S from Fluka; Chemicon (Temecula, CA, USA) and MTT from Atocel Co. (Trahütten, Graz, Austria).

MSCs isolation and culture

In this experimental study, MSCs originated from hBMs and hAMs were investigated. The BMs were

obtained from normal donors and the term placentas were obtained after caesarean section from healthy women. All samples were collected after informed consent. The AMs were prepared as previously described (Ahmadi et al. 2015). Briefly, AMs were mechanically peeled from the placenta and washed with phosphate buffer solution (PBS) three times. The AMs were then cut into small pieces and incubated with collagenase type IV solution (1 mg/mL) for 4 h at 37 °C, in the presence of 5% CO₂. The solution was then filtrated through a 70 µm cell strainer to remove any undigested fragments. The passed cells after washing with PBS were resuspended in low glucose-DMEM medium (supplemented with 10% FBS, 100 IU/mL penicillin and 100 µg/mL streptomycin) and placed in a T75 cell culture flask. After 24 h of incubation at 37 °C in a humidified atmosphere with 5% CO₂, non-adherent cells and the small digested residues were removed through washing with PBS while adherent cells were allowed to proliferate for more days. The medium was replaced two to three times a week. When adherent cells reached 90% confluence, they were detached by treatment with 0.25% Tyrosin-EDTA for 2–5 min at 37 °C, washed and transferred into other flasks for further culturing.

Proliferation assay

To evaluate viability and proliferation rate of MSCs, different experiments including trypan blue exclusion and MTT assay, in addition to the evaluation of growth curve and doubling time of the cultured cells were applied. The detailed description of these techniques is available in the supplementary methods.

Flow cytometric analyses of MSCs surface antigens

To characterize the expression of specific surface antigens on BM-MSCs by flow cytometry, mouse anti-human monoclonal antibodies against CD29, CD73, CD166 and CD105 [all conjugated with phycoerythrin (PE)], as well as against CD14, CD34, CD44, CD45 and CD90 [all conjugated with fluorescein isothiocyanate (FITC)], were applied to the third passage of these cells. In addition, isotype matched mouse mAbs conjugated with FITC and PE were used as negative controls to determine background staining. Sample acquisition was performed by a Partec PAS III flow

cytometer (Partec GmbH, Münster, Germany) and flow cytometric data were then analyzed using FlowJo software (FlowJo Tree Star, Inc., Ashland, OR, USA).

In vitro osteogenic differentiation

The osteoblasts were differentiated from MSCs as previously described (Hosseini et al. 2017). Briefly, to accelerate the osteogenesis process, the suspension of 6×10^4 MSCs in a low glucose- DMEM medium (supplemented with 10% FBS) was placed in a collagen (12 $\mu\text{g/mL}$) coated 24-well cell culture plate. When the cells reached 100% confluence, they were washed, detached, and resuspended in an osteogenic differentiation medium while the medium was half-changed every 72 h. The suspension of MSCs in low glucose-DMEM medium (supplemented with 10% FBS) was also applied as a negative control.

Alkaline phosphatase (ALP) activity

Osteoblasts express high level of ALP enzyme involved in the mineralization of bone matrix. To assess the existence of this enzyme, we used ALP staining which shows the earliest differentiation time. In the case of being positive, the cytoplasm is seen dark-purple. For this purpose, after 3, 5, 7 and 10 days of cultivation, the cells were washed with PBS and fixed with cold methanol. The cells were then covered with SIGMA FAST BCIP/NBT substrate and finally visualized by an inverted microscope. The substrate stains cells dark purple when ALP is present. Furthermore, the ALP activity of detached cells on day 10 of osteogenic induction was examined with substrate (p-nitrophenylphosphate) in a colorimetric assay and the optical density was read at 400 nm.

Mineralized matrix formation

The differentiation of MSCs into osteoblasts is associated with mineralization leading to the deposition of calcium in cytoplasm. These alternations are usually assessed by alizarin red S staining. In the case of being positive for alizarin red S, the osteoblast cytoplasm is orange-red whereas MSCs as the negative control are not stained with this dye. To perform this staining, the osteoblasts were fixed with cold ethanol (70%) and after washing with PBS 2 times, the

cells were covered with alizarin red S solution that specifically stains calcium deposits in orange-red.

Statistical analysis

All results are expressed as mean $\pm$ SD. Mann–Whitney U test was used to determine statistical difference between MSCs originated from hAMs and hBMs in the expression of specific surface antigens and hematopoietic markers. Data were analyzed by Graphpad Prism, version 6.07 (Graphpad Software Inc., San Diego, CA, USA). *P* values < 0.05 were considered as statistically significant.

Results

Morphology of MSCs

As a general characteristic property of MSCs, hAM-MSCs showed plastic adherence which was comparable to hBM-MSCs. The third passage of the hAM-MSCs and hBM-MSCs was frozen immediately in liquid nitrogen. The viability of post-thawing AM-MSCs and BM-MSCs was 80 ± 6.1 and $85 \pm 8\%$ ($n = 5$), respectively. Furthermore, the thawed hAM-MSCs illustrated morphological characteristics indistinguishable from the thawed hBM-MSCs.

Proliferation study

Figure 1A illustrates the increase in the number of viable adherent cells, measured by sorting of trypan blue negative cells, as curve growth. As shown in Fig. 1B, during the log phase of growth, proliferation of hBM-MSCs and hAM-MSCs expressed as doubling time, was 49 ± 6.3 and 35 ± 9 h at passage 3 and 56 ± 14 and 33 ± 11 h at passage 5, respectively. There was no significant difference in the doubling times of MSC passages 3 and 5 of the log-phase of growth. However, we found a significantly ($P < 0.05$) lower doubling time of hAM-MSCs compared to hBM-MSCs, which refers to higher proliferation rate.

Immunophenotyping of hMSCs

To assess the expression of specific and hematopoietic markers, the passages 3 of the MSCs originated from either hBMs or hAMs were subjected to flow

Fig. 1 Growth pattern and doubling time of MSCs in culture. Growth curve at passage 3 for the hBM-MSCs and hAM-MSCs (A) and doubling times at passages 3 and 5 during cell culture

for the BM-MSCs and AM-MSCs (B). *d* day, *DT* doubling time, *hAM* human amnion membrane, *hBM* human bone marrow, *MSCs* mesenchymal stem or stromal cells, *P* passage

Table 1 Adhesion molecules and hematopoietic regulator receptors expressed by MSCs

	Common name	CD locus	Detection
Adhesion molecules	ALCAM	CD166	Pos
	Hyaluronate receptor	CD44	Pos
Integrins	VLA-β chain	CD29	Pos
	LPS receptor	CD14	Neg
	Hematopoietic progenitor cell antigen	CD34	Neg
Additional markers	Leukocyte common antigen	CD45	Neg
	Thy-1	CD90	Pos
	Endoglin	CD105	Pos
	5' terminal nucleotidase	CD73	Pos

MSCs mesenchymal stem or stromal cells

cytometric analysis, as described in Materials and Methods. As shown in Table 1, MSCs originated from both amnion membrane and bone marrow were characterized by their expression of adhesion molecules and hematopoietic regulator receptors. According to the results, MSCs strongly expressed specific surface antigens of CD90, CD44, CD166, CD105, CD29 and CD73; whereas they did not express hematopoietic markers including CD45, CD14 and CD34. Figure 2A and Table 2 compare the expression of the aforementioned markers on hAM-MSCs and hBM-MSCs. In addition, Fig. 2B shows the representative histograms of flow cytometric analysis of hAM-MSCs at passage 3.

Characterization of differentiation of MSCs into osteoblast cells

In the first 3–5 days after adding osteogenic induction medium to MSCs, the appearance of differentiated cells was changed from long spindles to a cuboidal morphology. Furthermore, the assessment of the expression of ALP and cytoplasmic deposition of calcium in osteoblasts on days 3, 5, 7 and 10 of cultivation showed that the first positive results for the cells derived from both sources of hBM-MSCs and hAM-MSCs were obtained on day 5 of culture. As shown in Fig. 3A–D, hAM-MSCs differentiated to osteoblasts were positive for the expression of ALP and also for calcium deposits whereas undifferentiated

Fig. 2 Flow cytometric analysis of MSCs. The human BMs obtained from the normal donors and human placenta tissues were collected from the healthy women after caesarean section. The passage 3 of hAM-MSCs as well as of BM-MSCs was subjected to flow cytometry analysis to assess the expression of specific and hematopoietic markers. In addition, isotype matched mouse mAbs were used as negative controls to determine background staining. **A** Compares the expression of the markers between MSCs originated from either hAMs or hBMs ($n = 5$), **B** illustrates the representative histograms of flow cytometric analysis of AM-MSCs at passage 3. *hAM* human amnion membrane, *hBM* human bone marrow, *MSCs* mesenchymal stem or stromal cells

Table 2 The mean expression of specific and hematopoietic markers on MSCs derived from either hAMs or hBMs (n = 5)

Sources/markers	Adhesion molecules		Integrin	Additional markers					
	CD166	CD44	CD29	CD90	CD105	CD73	CD14	CD34	CD45
hAM-MSCs	82 ± 7.8	87 ± 7.9	89 ± 9.3	91 ± 6	80 ± 9	92 ± 7.3	1.3 ± 0.58	0.75 ± 0.53	1.8 ± 1.1
hBM-MSCs	91 ± 7.4	94 ± 3.9	92 ± 6.9	81 ± 13	88 ± 8.6	88 ± 6.5	2 ± 0.82 1	1.1 ± 0.42 1	3 ± 1.5

hAM human amnion membrane, *hBM* human bone marrow, *MSCs* mesenchymal stem or stromal cells

Fig. 3 Detection of osteogenic differentiation of MSCs. The osteoblasts differentiated from the representative hAM-MSCs on day 5 of induction, were stained bright orange-red using alizarin red S due to deposition of calcium (A). Using BCIP/NBT substrate, osteoblasts were also stained positive for ALP (dark purple) (B). As negative controls, undifferentiated hAM-MSCs are colorless, that shows no calcium deposits (C), negative ALP activity in these cells (D). Images were taken with an inverted microscope at 40×. **E** Compares the ALP activity of

osteogenic induced MSCs on day 10 of cultivation compared to non-induced MSCs. ALP alkaline phosphatase, *hAM* human amnion membrane, *hBM* human bone marrow, *MSCs* mesenchymal stem or stromal cells. * $p < 0.05$ shows statistical difference between the ALP activity of osteogenic induced MSCs and non-induced MSCs as well as not significant (ns) displays no statistical difference between the ALP activity of osteogenic induced MSCs (osteoblasts) obtained from hBMs and hAMs. Scale bar = 100 μm

hAM-MSCs (as a control group) were negative for both stainings. Moreover, Fig. 3E shows significantly higher ALP activity of osteogenic induced MSCs (osteoblasts) on day 10 of cultivation compared to non-induced MSCs. These results confirmed the differentiation of MSCs into osteoblasts. As shown in this figure, there was no significant difference between osteoblasts differentiated from human amnion membrane and bone marrow in terms of ALP activity.

Discussion

For decades, BM has been recognized as a most reliable source of MSCs applicable for cell therapy, regenerative medicine and different therapeutics or research-based studies. However, the use of this source of MSCs is usually hampered by several disadvantages mainly including in invasive procedures of BM collection, sample heterogeneity, suitable volunteers availability for BM donation and

ethical issues. Therefore, given the fact that MSCs is also technically obtainable from other sources, scientists focused to find more appropriate alternative sources of MSCs. As a highly abundant and readily available tissue, hAM is considered to be a suitable source of MSCs with the advantages of fewer ethical issues, low immunogenicity and anti-inflammatory properties.

In the present study, in order to find an appropriate alternative source for MSCs rather than BM, we first introduced a valid method of MSCs isolation from hAMs. We characterized isolated cells as MSCs and then compared their specific immunophenotypic criteria with those of MSCs obtained from BMs. To further evaluate MSCs characteristics, we tried to expand and differentiate hAM-MSCs into osteoblasts. We also compared hAM-MSCs osteogenic capacity with those of hBM-MSCs. While we showed comparable immunophenotypic criteria for MSCs originated from these two sources, we also showed that their osteogenic capacity were comparable.

In our study, hBM-originated MSCs were characterized by strong expression of CD90, CD44, CD166, CD105, CD29 and CD73. Several studies also used this immunophenotypic profile as specific surface antigens that represent MSCs. Our MSCs also did not express hematopoietic markers of CD45, CD14 and CD34 as already shown in other studies (Kilic et al. 2007; Mafi et al. 2011; Shen et al. 2012). Expanded hBM-MSCs were also successfully differentiated into the osteoblasts. We showed ALP activity of the cells from 5 days after expansion. Osteoblasts express high level of alkaline phosphatase enzyme involved in the mineralization of bone matrix. Osteogenic capacity of these cells was also confirmed by alizarin red S, which stains cytoplasmic calcium deposits due to mineralization. Our data are comparable with other studies that showed similar osteogenic capacity for MSCs originated from BM (Post et al. 2008; Wang et al. 2015). After successful preparation and characterization of our BM-originated MSCs, we tried to isolate MSCs from amnion membrane. We then evaluated their characteristics by using the aforementioned immunophenotyping and osteogenic capacities. So far, several groups who also isolated MSCs from amnion membrane characterized their cells by the evident expression of the mesenchymal markers including CD73, CD90, CD105, CD10, CD13, CD29, CD44, CD49c, CD49d, CD49e, CD54,

CD140b, CD166, CD349, STRO-1 and HLA-ABC (Lindenmair et al. 2012).

There are some studies that also confirmed differentiation capacities of hMSCs. Similar to our study, Shu et al. evaluated the induced differentiation of hAM-MSCs into osteoblasts. After 15 days of culture of the cells in osteogenic medium, they showed ALP, osteocalcin and osteopontin expression as well as secretion of type I collagen (Shu et al. 2011). However, in our study the significant expression of ALP was detected from day 5 of culturing and the alizarin red S staining also became positive from day 5. ALP staining was continued by day 10, when the cells started to be differentiated into osteocytes exhibiting obvious mineralized nodules.

We also evaluated the post-thawing viability of hAM-MSCs, which was around $80 \pm 6.1\%$ while these cells appropriately kept their osteogenic capacities as already mentioned. In a similar study Moon et al. also found post-thawing viability of $84.3 \pm 3.2\%$ for amnion-derived MSCs which maintained their ability to differentiate into osteoblasts, adipocytes and chondrocytes under appropriate culture conditions using von Kossa, oil red O and alcian blue staining, respectively (Moon et al. 2008).

We also showed the higher rate of proliferation for hAM-MSCs than that of hBM. This finding suggests shorter culturing time for hAM-MSCs to obtain sufficient population of MSCs applicable for therapeutic approaches. Our data are similar to a study that showed higher growth rate of umbilical cord blood-MSCs compared to adult BM-MSCs (Jin et al. 2013).

Most recently, to study the effects of hAM-MSCs on the proliferation and differentiation of hBM-MSCs, Wang et al. have applied a transwell coculture of hAM-MSCs and hBM-MSCs in basal medium. To monitor hBM-MSCs proliferation, they evaluated 5-ethynyl-2'-deoxyuridine (EdU) expression of the cells with immunofluorescence testing. In addition, they examined osteoblastic differentiation and mineralization by ALP activity substrate assays, alizarin red S staining and RT-PCR analysis of early hBM-MSCs osteogenic marker expression (mRNA expression of ALP, Runx2, and Osteocalcin) during cell culturing. They compared all these markers in a co-culture system with single control cultures and concluded that hAM-MSCs can promote proliferation and osteogenic differentiation of BM-MSCs (Wang et al. 2015, 2016).

However, their finding is complicated by the fact that hAM-MSCs per se can differentiate into osteoblasts (Manochantr et al. 2010; Shu et al. 2011; Lindenmair et al. 2012) as the osteogenic capacity was one of the first capabilities being demonstrated for hAMSC (In't Anker et al. 2004; Portmann-Lanz et al. 2006). Therefore, in our study we decided to directly compare the immunophenotypic characteristics and osteogenic capacity of hAM-MSCs with those of hBM-MSCs. In our study, hAM-MSCs showed morphological characteristics indistinguishable from those of hBM-MSCs. In addition, as a general characteristic property for MSCs, their plastic adherences were similar to each other. During expansion, the proliferation rate and viability of hAM-MSCs were not significantly different from hBM-MSCs. We also found the comparable expression of specific antigens of these two MSCs including CD90, CD44, CD166, CD105, CD29 and CD73. Intriguingly, using ALP and alizarin red S staining, either hAM-MSCs or hBM-MSCs differentiation into osteoblasts successfully occurred from day 5 of culturing, which may confirm the appropriate osteogenic capacity of MSCs.

Conclusion

Taken together, our study showed comparable characteristic immunophenotypes between hMSCs originated from amnion membrane and bone marrow. We also revealed that amnion membrane derived MSCs also have osteogenic capacities similar to BM-MSCs. Notably, higher proliferation rate observed for hAM-MSCs suggests a shorter culturing time to provide sufficient number of MSCs accessible for different applications. Therefore, amnion membrane-derived MSCs per se can be considered as an easily accessible and reliable source of MSCs especially applicable for bone tissue engineering, regenerative medicine or other therapeutic approaches.

Acknowledgements This work was part of Dr. Hosseini's approved project (No. 1390-01-33-1516) supported by Iranian blood transfusion organization and High Institute for Research and Education in Transfusion Medicine in Iran. The authors declare no conflict of interests.

References

- Ahmadi M, Hosseini E, et al (2015) The role of amnion membrane-derived mesenchymal stem cells on differentiation and expansion of natural killer cell progenitors originated from umbilical cord blood mononuclear cells. *Biotechnol Health Sci* 2:36–39
- Barry FP (2003) Mesenchymal stem cell therapy in joint disease. *Novartis Found Symp* 249:86–96 (**discussion 96–102, 170–104, 239–141**)
- Bernardo ME, Locatelli F (2016) Mesenchymal stromal cells in hematopoietic stem cell transplantation. *Methods Mol Biol* 1416:3–20
- Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 213:341–347
- Caplan AI (2009) Why are MSCs therapeutic? New data: new insight. *J Pathol* 217:318–324
- Celebi B, Mantovani D et al (2011) Irradiated mesenchymal stem cells improve the ex vivo expansion of hematopoietic progenitors by partly mimicking the bone marrow endosteal environment. *J Immunol Methods* 370:93–103
- Chen WM, Chen ZX et al (2008) Osteoblasts from patients with myelodysplastic syndrome express multiple cytokines and support hematopoietic progenitor cell survival in vitro. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 16:78–83
- Conrad C, Zeindl-Eberhart E et al (2008) Alkaline phosphatase, glutathione-S-transferase-P, and cofilin-1 distinguish multipotent mesenchymal stromal cell lines derived from the bone marrow versus peripheral blood. *Stem Cells Dev* 17:23–27
- de Barros AP, Takiya CM et al (2010) Osteoblasts and bone marrow mesenchymal stromal cells control hematopoietic stem cell migration and proliferation in 3D in vitro model. *PLoS ONE* 5:e9093
- Deans RJ, Moseley AB (2000) Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol* 28:875–884
- Ding DC, Chang YH et al (2015) Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. *Cell Transplant* 24:339–347
- Du X, Yuan Q et al (2016) Endometrial mesenchymal stem cells isolated from menstrual blood by adherence. *Stem Cells Int* 2016:3573846
- Fajardo-Orduna GR, Mayani H et al (2015) Hematopoietic support capacity of mesenchymal stem cells: biology and clinical potential. *Arch Med Res* 46:589–596
- Fajardo-Orduna G, Mayani H et al (2016) Bone marrow mesenchymal stromal cells from clinical scale culture: in vitro evaluation of their differentiation, hematopoietic support and immunosuppressive capacities. *Stem Cells Dev* 25:1299–1310
- Fujita S, Toguchida J et al (2008) Clonal analysis of hematopoiesis-supporting activity of human mesenchymal stem cells in association with Jagged1 expression and osteogenic potential. *Cell Transpl* 17:1169–1179
- He Q, Wan C et al (2007) Concise review: multipotent mesenchymal stromal cells in blood. *Stem cells (Dayton, Ohio)* 25:69–77
- Herzog EL, Chai L et al (2003) Plasticity of marrow-derived stem cells. *Blood* 102:3483–3493

- Honmou O, Onodera R et al (2012) Mesenchymal stem cells: therapeutic outlook for stroke. *Trends Mol Med* 18:292–297
- Hoogduijn MJ, Crop MJ et al (2007) Human heart, spleen, and perirenal fat-derived mesenchymal stem cells have immunomodulatory capacities. *Stem Cells Dev* 16:597–604
- Hosseini E, Ghasemzadeh M et al (2017) Ex vivo expansion of CD3 depleted cord blood-MNCs in the presence of bone marrow stromal cells; an appropriate strategy to provide functional NK cells applicable for cellular therapy. *Stem Cell Res* 19:148–155
- Huang XB, Liu T et al (2006) Osteoblasts differentiated from human marrow bone mesenchymal stem cells support hematopoietic stem/progenitor cells from umbilical cord blood. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 14:552–556
- Hung SC, Chen NJ et al (2002) Isolation and characterization of size-sieved stem cells from human bone marrow. *Stem Cells* 20:249–258
- In't Anker PS, Scherjon SA et al (2004) Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells* 22:1338–1345
- Jackson WM, Nesti LJ et al (2012) Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. *Stem Cells Transl Med* 1:44–50
- Jin HJ, Bae YK et al (2013) Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. *Int J Mol Sci* 14:17986–18001
- Jo Y, Lee H et al (2007) Isolation and characterization of postnatal stem cells from human dental tissues. *Tissue Eng* 13:767–773
- Jung Y, Wang J et al (2005) Cell-to-cell contact is critical for the survival of hematopoietic progenitor cells on osteoblasts. *Cytokine* 32:155–162
- Kilic E, Ceyhan T et al (2007) Evaluation of differentiation potential of human bone marrow-derived mesenchymal stromal cells to cartilage and bone cells. *Acta Orthop Traumatol Turc* 41:295–301
- Klein C, Strobel J et al (2013) Ex vivo expansion of hematopoietic stem- and progenitor cells from cord blood in coculture with mesenchymal stroma cells from amnion, chorion, Wharton's jelly, amniotic fluid, cord blood, and bone marrow. *Tissue Eng Part A* 19:2577–2585
- Kuo Ching Chao KFC (2008) Islet-like clusters derived from mesenchymal stem cells in Wharton's jelly of the human umbilical cord for transplantation to control type 1 diabetes. Public Library of Science
- Li Q, Zhou X et al (2013) In vivo tracking and comparison of the therapeutic effects of MSCs and HSCs for liver injury. *PLoS ONE* 8:e62363
- Lindenmair A, Hatlapatka T et al (2012) Mesenchymal stem or stromal cells from amnion and umbilical cord tissue and their potential for clinical applications. *Cells* 1:1061–1088
- Madrigal M, Rao K et al (2014) A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. *J Transl Med* 12:260
- Mafi P, Hindocha S et al (2011) Adult mesenchymal stem cells and cell surface characterization: a systematic review of the literature. *Open Orthop J* 5:253–260
- Manochantr S, Tantrawatpan C et al (2010) Isolation, characterization and neural differentiation potential of amnion derived mesenchymal stem cells. *J Med Assoc Thai* 93:S183–S191
- Miura Y (2013) Regulation of hematopoiesis by mesenchymal stem cells. *Rinsho Ketsueki* 54:431–435
- Mobasheri A, Csaki C et al (2009) Mesenchymal stem cells in connective tissue engineering and regenerative medicine: applications in cartilage repair and osteoarthritis therapy. *Histol Histopathol* 24:347–366
- Moon JH, Lee JR et al (2008) Successful vitrification of human amnion-derived mesenchymal stem cells. *Hum Reprod* 23:1760–1770
- Neve A, Corrado A et al (2011) Osteoblast physiology in normal and pathological conditions. *Cell Tissue Res* 343:289–302
- Nuschke A (2014) Activity of mesenchymal stem cells in therapies for chronic skin wound healing. *Organogenesis* 10:29–37
- Ochsenbein-Kolble N, Bilic G et al (2003) Inducing proliferation of human amnion epithelial and mesenchymal cells for prospective engineering of membrane repair. *J Perinat Med* 31:287–294
- Ogura N, Kawada M et al (2004) Differentiation of the human mesenchymal stem cells derived from bone marrow and enhancement of cell attachment by fibronectin. *J Oral Sci* 46:207–213
- Oh W, Kim DS et al (2008) Immunological properties of umbilical cord blood-derived mesenchymal stromal cells. *Cell Immunol* 251:116–123
- Pittenger MF, Mackay AM et al (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147
- Pontikoglou C, Deschaseaux F et al (2011) Bone marrow mesenchymal stem cells: biological properties and their role in hematopoiesis and hematopoietic stem cell transplantation. *Stem Cell Rev* 7:569–589
- Portmann-Lanz CB, Schoeberlein A et al (2006) Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. *Am J Obstet Gynecol* 194:664–673
- Post S, Abdallah BM et al (2008) Demonstration of the presence of independent pre-osteoblastic and pre-adipocytic cell populations in bone marrow-derived mesenchymal stem cells. *Bone* 43:32–39
- Ramakrishnan A, Torok-Storb B et al (2013) Primary marrow-derived stromal cells: isolation and manipulation. *Methods Mol Biol* 1035:75–101
- Ren H, Sang Y et al (2016) Comparative analysis of human mesenchymal stem cells from umbilical cord, dental pulp, and menstrual blood as sources for cell therapy. *Stem Cells Int* 2016:3516574
- Schmal O, Seifert J et al (2016) Hematopoietic stem and progenitor cell expansion in contact with mesenchymal stromal cells in a hanging drop model uncovers disadvantages of 3D culture. *Stem Cells Int* 2016:4148093
- Sensebe L, Bourin P (2009) Mesenchymal stem cells for therapeutic purposes. *Transplantation* 87:S49–S53
- Shen JL, Huang YZ et al (2012) Effectiveness of human mesenchymal stem cells derived from bone marrow cryopreserved for 23–25 years. *Cryobiology* 64:167–175

- Shiozawa Y, Takenouchi H et al (2008) Human osteoblasts support hematopoietic cell development in vitro. *Acta Haematol* 120:134–145
- Shu J, Guo L et al (2011) Experimental study on the induced differentiation of human amnion mesenchymal cells into osteoblasts. *Zhonghua zheng xing wai ke za zhi = Zhonghua zhengxing waikē zazhi = Chin J Plast Surg* 27:362–367
- Tae SK, Lee SH et al (2006) Mesenchymal stem cells for tissue engineering and regenerative medicine. *Biomed Mater* 1:63–71
- Verfaillie CM (1998) Adhesion receptors as regulators of the hematopoietic process. *Blood* 92:2609–2612
- Walenda T, Bokermann G et al (2011) Synergistic effects of growth factors and mesenchymal stromal cells for expansion of hematopoietic stem and progenitor cells. *Exp Hematol* 39:617–628
- Wang L, Li ZY et al (2015a) Dynamic expression profiles of marker genes in osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. *Chin Med Sci J* 30:108–113
- Wang Y, Yin Y et al (2015b) Human amnion mesenchymal stem cells promote proliferation and osteogenic differentiation in human bone marrow mesenchymal stem cells. *J Mol Histo* 46:13–20
- Wang Y, Jiang F et al (2016a) Human amnion-derived mesenchymal stem cells promote osteogenic differentiation in human bone marrow mesenchymal stem cells by influencing the ERK1/2 signaling pathway. *Stem Cells Int* 2016:4851081
- Wang Y, Yu X et al (2016b) Liver-derived human mesenchymal stem cells: a novel therapeutic source for liver diseases. *Stem Cell Res Ther* 7:71
- Wei X, Yang X et al (2013) Mesenchymal stem cells: a new trend for cell therapy. *Acta Pharmacol Sin* 34:747–754
- Yang ZX, Han ZB et al (2013) CD106 identifies a subpopulation of mesenchymal stem cells with unique immunomodulatory properties. *PLoS ONE* 8:e59354
- Zannettino AC, Paton S et al (2008) Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo. *J Cell Physiol* 214:413–421
- Zhao K, Liu Q (2016) The clinical application of mesenchymal stromal cells in hematopoietic stem cell transplantation. *J Hematol Oncol* 9:46