Comparative Analysis of Adult Mesenchymal Stem Cells Derived from Adipose, Bone-Marrow, Placenta, and Umbilical Cord Tissue

Tiana Tonrey MS, Jim Musick PhD, Richard Dean BS, Krutika Patel

Abstract

Mesenchymal stromal/stem cells (MSCs) have the potential to repair and regenerate damaged tissues, making them attractive candidates for cell-based therapies. Expanded and well-characterized MSCs have application in regenerative medicine and have been used in several clinical trials including treatment for osteoarthritis and other conditions. Here, we provide results of a comparative study of purified and expanded MSCs from adipose, bone-marrow, placenta, and umbilical cord involving determination of phenotype by flow cytometry analysis, cellular potency by quantitative assessment of mitochondrial function and immunosuppression, and cellular function by quantitative assessment of cell migration and proliferation. Our results show comparable phenotypic profiles, morphology, expansion in cell culture and adipogenic, osteogenic and chondrogenic differentiation. Potency measures of mitochondrial/ immunosuppressive capacity and additional cellular function assays show differences suggesting biological advantages of umbilical MSCs.

Introduction

Mesenchymal stem cells (MSCs) are multipotent, non-differentiated adult stem cells capable of self-renewal, proliferation, conversion into differentiated cells as well as the regeneration of tissues. MSC-based regenerative medicine offers novel therapies for patients with injuries, end-stage organ failure, degenerative diseases, and several other medical conditions. Transplanted MSCs have shown potential therapeutic benefits and safety in myocardial, musculoskeletal, neurological, autoimmune disorders, and several other disorders (Carralho E, et al. Regen. Med. 2015; 10:1025; Freitag J, et al. BMC Musculoskeletal Disorders 2016; 17: 230; Neirinckx V, et al. Stem Cell Trans. Med 2013; 2: 284; Munir H and McGettrick, HM Stem Cells Dev. 2015; 24:2091). MSCs are isolated from several tissues including lipoaspirates, perinatal tissues, cord blood, teeth, etc. and have considerable capacity for *in vitro* expansion and broad regenerative potential. These properties make MSCs attractive candidates for cell-based therapies.

In the United States, stem cells derived from expanded MSCs are presently excluded from clinical use since the FDA classifies this product as a drug requiring a New Drug Application (NDA) or equivalent submission, multiple clinical trials, and approval prior to marketing. No MSC-based therapies are yet approved for clinical application in the US while hematopoietic stem cells are FDA-approved for clinical use. The European Medicines Agency has recently approved allogeneic MSCs (Alifosel™) derived from adipose-tissue for treatment of a type of Crohn's disease. Other countries have different regulatory requirements for commercial approval of stem cell therapies.

Clinical trials based on expanded MSCs are common internationally, although there are variations in the degree of regulation including the requirements for adherence to cGMP standards.. Here, we report on phenotypic and functional characterization of purified and expanded MSCs from adipose, bone-marrow, placenta, and umbilical cord. Our results show comparable growth and tri-lineage differentiation performance while umbilical cord MSCs display enhanced potency, cellular functions and capacity for differentiation into neural stem cells.

Materials and Methods

MSC Purification and Expansion

AD-MSCs: Adipose lipoaspirate (AD) was purchased from ZenBio (Cat. No. T-SQ-50mL) and was digested using collagenase. The cells were plated in TC-coated T-75 flasks (B.D. Falcon, Cat. No. 353136) in **MSC-Gro™** low serum medium (Vitro Biopharma, Cat. No. SC00B1) plated at 15,000/cm^2 viable MNCs for isolation and purification. This initial passage of the primary cell culture was referred to as passage 0 (P_0). Cells were maintained in media until they achieved 75%–90% confluency.

BM-MSCs: Bone marrow (BM) mesenchymal stem cells were purchased from ATCC (Cat. No. PCS-500-012) and were plated in TC-coated T75 flasks (B.D. Falcon, Cat. No. 353136) in **MSC-Gro™** low serum medium (Vitro Biopharma, Cat. No. SC00B1) plated at 5,000/cm^2 viable MSCs. Cells were maintained in media until they achieved 75%–90% confluency.

P-MSCs: Placenta (P) was donated from a healthy woman during a caesarean section at the end of gestation, with a signed informed consent. The cells were digested using collagenase and plated in TC-coated T75 flasks (B.D. Falcon, Cat. No. 353136) in **MSC-Gro™** low serum medium (Vitro Biopharma, Cat. No. SC00B1) plated at 15,000/cm^2 viable MNCs for isolation and purification. This initial passage of the primary cell culture was referred to as passage 0 (P_0). Cells were maintained in media until they achieved 75%–90% confluency.

UC-MSCs: Umbilical cord (UC) was donated from a healthy woman during natural delivery at the end of gestation, with a signed informed consent. The cells were digested using collagenase and plated in TC-coated T75 flasks (B.D. Falcon, Cat. No. 353136) in **MSC-Gro™** low serum medium (Vitro Biopharma, Cat. No. SC00B1) plated at 15,000/cm^2 viable MNCs for isolation and purification. This initial passage of the primary cell culture was referred to as passage 0 (P_0). Cells were maintained in media until they achieved 75%–90% confluency.

AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs were expanded until passage 2 (P_2) and were analyzed for cell count, viability, flow cytometry, potency by quantitation of γ-IFN-induced IDO activity (Hong J, et al. 2014; 4:4645), cell-specific ATP, an independent measure of stem cell potency (Harper, H and Rich, IN, Methods Mol Biol. 2015;1235:33), and cell-based functionality by analysis of migration and proliferation. The phenotypic identity of MSCs was determined by the criteria published by the ISCT for the definition of a stem cell (Dominici M, et al. Cytotherapy. 2006; 8: 315).

Flow Cytometry Analysis

AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs were blocked with 1.5% HSA and stained with anti-human monoclonal antibodies for 1hr at room temperature against the following antigens: CD_{11b}, CD_{14}, CD_{19},

CD_{34}, CD_{45}, CD_{73} CD_{79a}, CD_{90}, CD_{105}, and HLA-DR (all PE conjugated). All cells had a non-stained sample for controls. PE-conjugated antibodies were purchased through Miltenyi Biotech. The flow cytometric analysis was performed on a Gallios cytofluorimeter (Beckman Coulter) and data were analyzed with Kaluoza software (CU Cancer Center Flow Cytometry Core Laboratory).

Immunomodulatory Potency Assay

The γ-IFN-induced indoleamine 2,3-dioxygenase (IDO) cell-based assay (Hong J, et al. 2014; 4:4645) was performed on the AD-MSCs, BM-MSCs, P-MSCs, and UC-MSC. Cells were plated at 5,000/cm^2 per well in TC-coated 96 well plates (Fisher Scientific, Cat. No. 130188) in **MSC-Gro™** low serum media (Vitro Biopharma, Cat. No. SC00B1) and incubated at 37°C for 24-hrs in a humidified, hypoxic atmosphere (1% O_2, 5%CO_2, 94% N_2). After a 24-hr incubation, cells were washed three times with 1x PBS (Fisher Scientific., Cat. No. BP655-1) and then cultured in quiescent, serum-free media (Vitro Biopharma, Cat. No. SC00B17) containing 10µM tryptophan (Sigma, Cat. No. T8941) and accelerating dosages of γ-IFN (0, 2.5, 5, 10 ng/ml). Cells were incubated at 37°C for a 72-hr period in humidified, reduced O_2 conditions (1% O_2, 5%CO_2, 94% N_2). Conditioned media was collected and plated in a new black 96 well plate (B.D. Falcon, Cat. No. 353219) with a kynurenine standard curve (0 to 100 µM) and stained with 0.5mM methylene blue for 60 min at 37°C. Fluorescence (E_x=365nm E_m> 430nm) was determined using a Modulus microtiter plate reader.

Mitochondrial Function Potency Assay

Cell potency was also measured by cell-specific ATP measurement in the luciferin-luciferase assay as previously used and validated for potency determination of hematopoietic stem cells (HSC) (Harper, H and Rich, IN, Methods Mol Biol. 2015;1235:33-48) and MSCs (Deskins D, et al.. Stem Cells Trans Med. 2013; 2:151). The adenosine triphosphate (ATP) assay was performed on the AD-MSCs, BM-MSCs, P-MSCs, and UC-MSC. Cells were plated at different concentrations per well in TC-coated 96 white well-plate (Fisher Scientific, Cat No. 130188) with an ATP standard curve in a quiescent serum-free media (Vitro Biopharma, Cat. No. SC00B17). Following culture in a humidified atmosphere of 1% O_2, 5%CO_2, 94% N_2 for 24 hours, 100 µl Cell Titer Glo™ (Promega, Cat. No. G9241) was added to all wells and incubated for 30-min at room temperature. Luminescence was determined on a Modulus microtiter plate reader.

Cell Migration Assay

One million cells of eachcell line were resuspended in 10mL **MSC-Gro™** serum free, quiescent medium (Vitro Biopharma Cat. No. SC00B17) containing 5µg/mL mitomycin C (Sigma, Cat. No. M4287) and incubated for 2hrs at room temperature with end-to-end agitation at 7 RPM. Cells were centrifuged (450 x g) for 7 minutes and washed out with PBS. Cells were resuspended in 1mL MSC-Gro™ low serum, complete medium (Vitro Biopharma Cat. No. SC00B1) and were plated at 25,000/well in TC-coated black 96 well cell culture plates (Thermo Fisher, Cat. No. 165305), containing cell seed stoppers (Platypus, Cat. No. CMAUFL4) to form a cell free zone and placed in 5%CO_2, 1%O_2, 94% N_2 at 37°C in a humidified chamber for 24hrs.

Cells were washed once with PBS then incubated in serum free, **MSC-Gro™** (Vitro Biopharma Cat. No. SC00B17) containing 5µM CellTracker Green CMFDA (Molecular Probes, Cat. No. C7025) at 37°C for 30 minutes. Cells were washed with serum free, **MSC-Gro™** (Vitro Biopharma Cat. No. SC00B17) and incubated for 30 minutes at 37°C. Cells were washed once with PBS and replaced with **MSC-Gro™** serum

free, quiescent medium (Vitro Biopharma Cat. No. SC00B17) containing different concentrations of activating agents. Substance P was purchase from Tocris Bioscience, (Cat. No. 1156) and used as a control. A TopSeal (PerkinElmer, Cat. No. 6050195) covered the plate and it was placed in a BioTek Cytation3 Imaging Reader. Kinetic data was acquired every 3hrs for 48hrs using GFP and bright field data acquisition. The gas phase throughout the acquisition of kinetic data was 5% O_2, 5% CO_2 with the balance nitrogen maintained by a BioTek CO_2 /O_2 gas controller. Images were saved as TIFF files and imported into the Image-J program to determine the area of the detection zone of post-migration wells in comparison with controls to calculate percent closure using imaging data. Values were imported into Origin 8.1 and graphed.

Cell Proliferation Assay

One hundred thousand cells/cell line were resuspended in 2mL MSC-Gro™ low serum, complete medium (Vitro Biopharma Cat. No. SC00B1) and plated at 5,000/well in tissue cultured black 96 well plates (Thermo Fisher, Cat. No. 165305) and placed in 5%CO_2, 1%O_2, 94%N_2 at 37°C in a humidified chamber for 24hrs.

Cells were washed 3x with PBS and replaced with **MSC-Gro™** serum free, quiescent media (Vitro Biopharma Cat. No. SC00B17) containing different concentrations of activating agents and growth factors. Cells were stained with 10μL PrestoBlue/well (Invitrogen, Cat. No. A13261) and incubated in the dark in a glove box at 1%O_2, 5%CO_2 at 37°C for 30 minutes. The plate was read using a FITC filter (Ex= 490nm, Em >510nm) for fluorescent intensities at Day 0 and 3 in a Modulus microtiter plate reader.

Differentiation of Neural Stem Cells

AD-MSCs, P-MSCs, and UC-MSCs were plated in a laminin T25 at 10k/cm^2 in neural differentiation media in 5%CO_2, 1%O_2, 94% N_2 at 37°C in a humidified chamber for 3 weeks with feeds twice a week. Cells were sub-cultured and plated into a laminin coated 24 well plate at 25k/cm^2 in neural stem cell media and placed in 5%CO_2, 1%O_2, 94%N_2 at 37°C in a humidified chamber for 72hrs. Cells were washed three times and fixed with 10% formalin for 20 minutes at room temperature. Cells were washed three times and permeabilize with 0.1% Triton-100x for 10 minutes at room temperature. Cell were washed three times and blocked with 5% goat-serum for 2 hours at room temperature. After incubation was complete, cells were stained with primary antibodies: Nestin, 3PDGH, GLAST, βIII-Tubulin, MAP2, and Neurofilament M (Santa Cruz Biotechnology). Cells were incubated overnight at 4°C. Cells were washed out three times and stained with secondary antibody (Anti-mouse IgG Alexa Fluor 488-conjugated) for 1 hour at room temperature. Cells were washed three times and stained with Hoecst 33528 for nuclear staining and read using a Cytation 3 (BioTek) cell analyzer for GFP and DAPI.

Results

Growth in Cell Culture

We initially compared the growth and expansion characteristics of AD-MSCs, BM-MSCs, P-MSCs and UC-MSCs following pass 2 in cell culture as described above. The results shown in Figure 1 at pass 2 show doubling time (Td) between 20 hours for UC-MSCs and 53 hours for BM-MSCs while MSC viability was relatively consistent at 85 to 95%. This shows comparable expansion at variable growth rates: UC-MSC>AD-MSC>P-MSC>BM-MSC. Similar results were seen in several replicates (n=4) of this protocol.
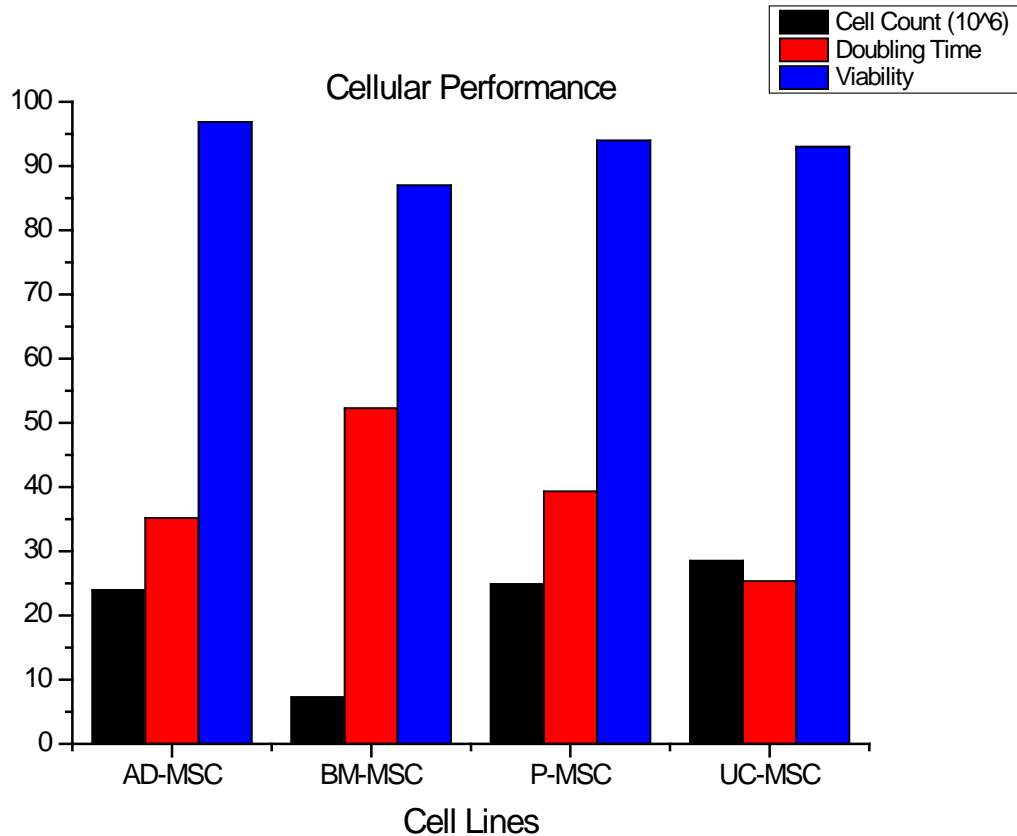.

Cellular Performance

Figure 1. Black bars are cell count, red bars are doubling times: $T \ln (C_f-C_i)/C_i$ where T is the time from subculture to detachment (Hrs), C_i is the initial cell count and C_f is the final cell count and blue bars are PI-determined viability as described in the Materials and Methods

Phenotypic Characterization of AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs

The isolated and expanded AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs were investigated for MSC phenotype at P_2 by staining for cell surface markers, which were detected using flow cytometry according to the ISCT standard (Dominici M, et al. Cytotherapy. 2006; 8: 315) and the results are shown in Table 1. The AD-MSCs and UC-MSCs expressed the typical MSC markers CD_{90}, CD_{73}, and CD_{105}. In addition, the cells showed low expression of hematopoietic markers $CD11_b$, CD_{14}, CD_{19}, CD_{34}, CD_{45}, and the MHC class II molecule HLA-DR. Similar results have been seen in several replicates (n=4). However, the P-MSCs expressed a high level of CD_{45}, possibly due to leukocyte contamination. The BM-MSCs also expressed higher levels of CD_{45} and CD_{79a}, possibly due to residual levels of B-cells.

Cell Surface Markers	ISCT Standard	UC-MSC	P-MSC	AD-MSC	BM-MSC
CD11b	Negative	Negative	Negative	Negative	Negative
CD14	Negative	Negative	Negative	Negative	Negative
CD19	Negative	Negative	Negative	Negative	Negative
CD34	Negative	Negative	Negative	Negative	Negative
CD45	Negative	Negative	Positive	Negative	Positive
CD73	Positive	Positive	Positive	Positive	Positive
CD79a	Negative	Negative	Negative	Negative	Positive
CD90	Positive	Positive	Positive	Positive	Positive
CD105	Positive	Positive	Positive	Positive	Positive
HLA-DR	Negative	Negative	Negative	Negative	Negative

Table 1. Summary results of flow cytometry analysis of UC-MSCs, P-MSCs, AD-MSCs & BM-MSCs compared to the ISCT standard definition of an MSC. Positive values are >90% and negative are < 5%

Immunomodulatory Potency Measures of AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs

To compare immunomodulatory properties of MSCs from various sources, the activation of IDO by exposure to γ-IFN was determined on an equivalent cellular basis (Fig. 2). The γ-IFN-induced IDO activity was quantified by the conversion of tryptophan to kynurenine. Maximal IDO activity at 10 ng/ml γ-IFN was ~4 fold greater in the isolated and expanded UC-MSCs versus other MSCs derived from other tissues. These results show greatest immunomodulatory cellular potency in expanded UC-MSCs followed by AD-MSCs, P-MSCs, and BM-MSCs. There was a significant difference in γ-IFN-induced IDO activity between the AD-MSCs, BM-MSCs, and P-MSCs compared to UC-MSCs with a p-value<0.005 by one-way ANOVA analysis (Graph Pad Prism™) of variance for significance of slope difference.
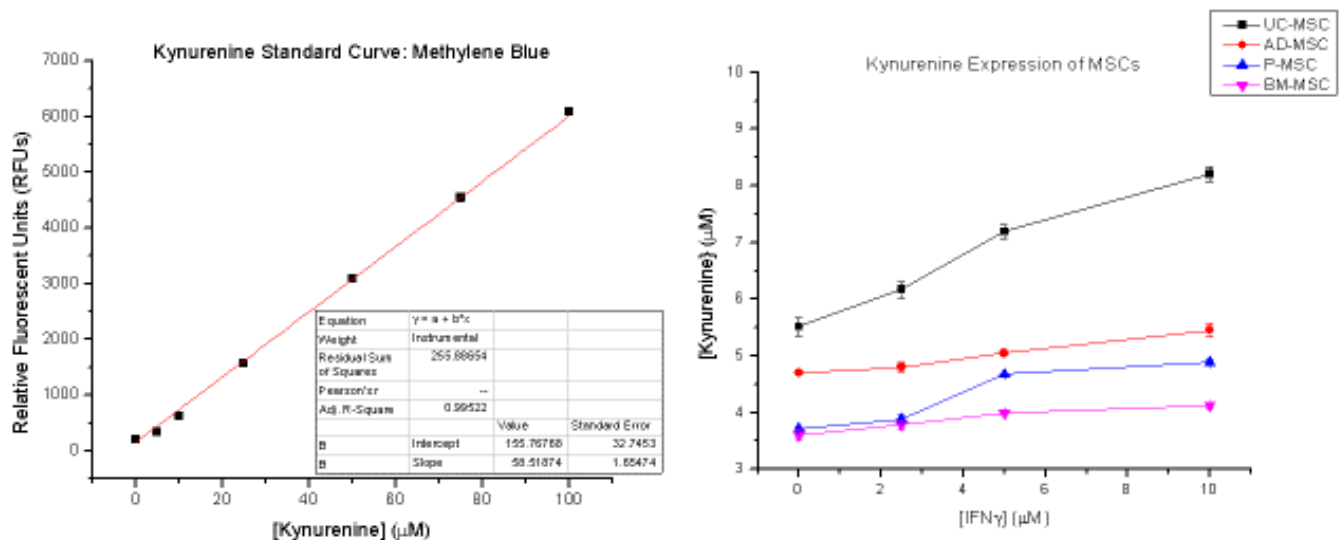
Figure 2. Immunomodulatory potency of UC-MSCs, AD-MSCs, P-MSCs and UC-MSCs by the γ-IFN induced IDO activity assay is shown above.

Mitochondrial Function Analysis of AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs

Potency was also measured by cell-specific ATP determination as previously used to determine potency of human HSCs & MSCs (Harper, H and Rich, IN, Methods Mol Biol. 2015;1235:33; Deskins D, et al.. Stem Cells Transl Med. 2013; 2:151) Relative luminescent units were converted to [ATP] using the ATP standard curve (Left panel, Fig. 3) and cellular ATP is shown as a function of cells per well (Right panel, Fig. 3). Cellular potency is measured by the slope of this relation (Harper, H and Rich, IN, Methods Mol Biol. 2015;1235:33; Deskins D, et al.. Stem Cells Transl Med. 2013; 2:151) and UC-MSCs showed greater potency than expanded AD-MSCs, BM-MSCs, and P-MSCs. There was a significant difference between the AD-MSCs, BM-MSCs, and P-MSCs compared to the isolated and expanded UC-MSCs with a p-value >0.05 by one-way ANOVA analysis (Graph Pad Prism™) of variance for significance of slope difference.
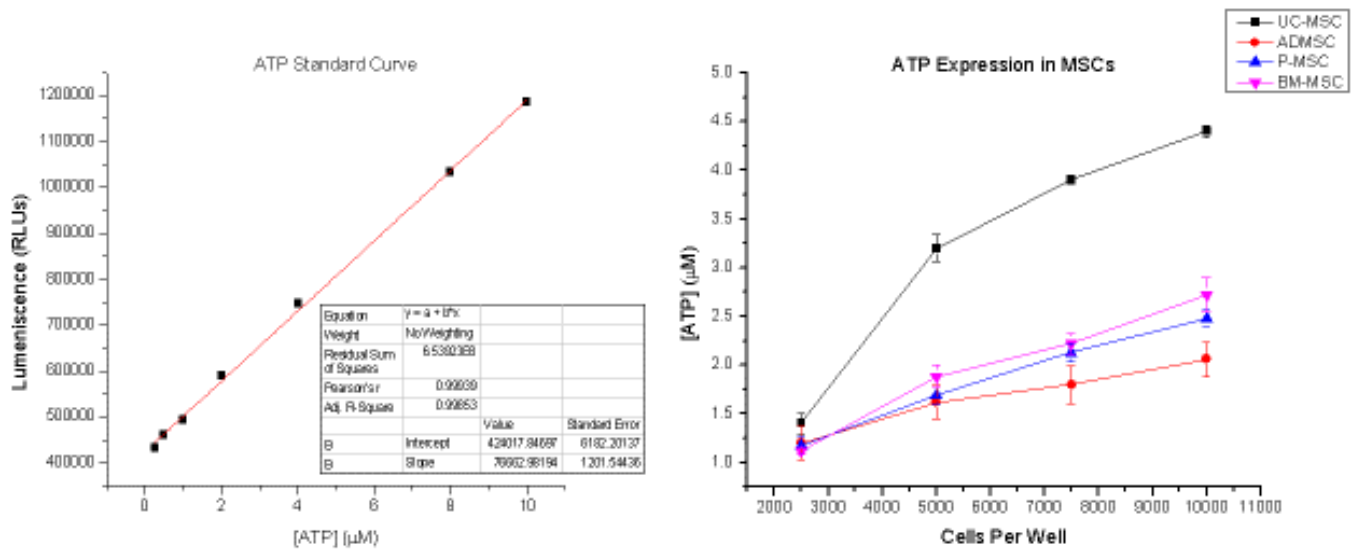

Figure 3. Umbilical cord MSCs showed a significantly higher cellular ATP-content than the other AD-MSCs, P-MSCs, & BM-MSCs.

Comparison of Cell Migration by AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs

Since MSCs are well known to migrate to sites of inflammation, injury and to cancer stem cells, we compared the migration of AD-MSCs, P-MSCs, BM-MSCs and UC-MSCs in response to exposure to Substance P, a multi-functional neuropeptide. The results show in Figure 4 show the relative migration measured as a per-cent closure of the occluded plate region following exposure to 50 pg/ml Substance P. UC-MSCs showed greatest closure at 50 pg/mL substance P (~40% closure), while AD-MSC, P-MSC, and BM-MSC had a closure between 5-15%. These results were seen in several replicates (n=4).
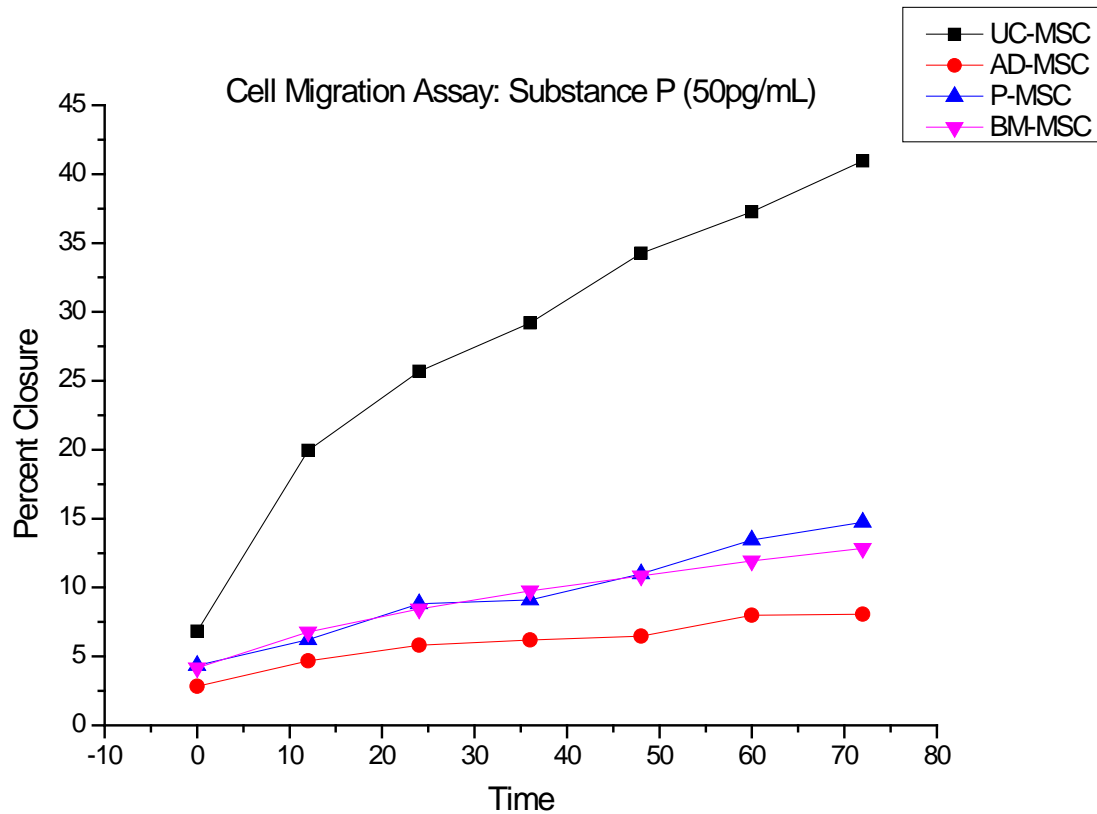
Figure 4. Comparison of migration by AD-MSCs, P-MSCs, BM-MSCs & UC-MSCs into cell-free regions. Migration was determined as described in the Materials and Methods and the measured % closure of the occluded region is plotted as a function of time after exposure to 50 pg/ml Substance P.

Cell Proliferation Analysis of AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs

We also compared proliferation capacity of AD-MSCs, P-MSCs, BM-MSCs and UC-MSCs by quantifying cellular redox activity by a well-validated resazurin-based fluorometric assay. Figure 5 shows the results of the comparison of proliferation by AD-MSCs, P-MSCs, BM-MSCs and UC-MSCs. The relative fluorescent difference at day 1 and 3 using Presto Blue as shown as a function of FBS content in a serum-free medium. These results were seen in several replicates (n=4).

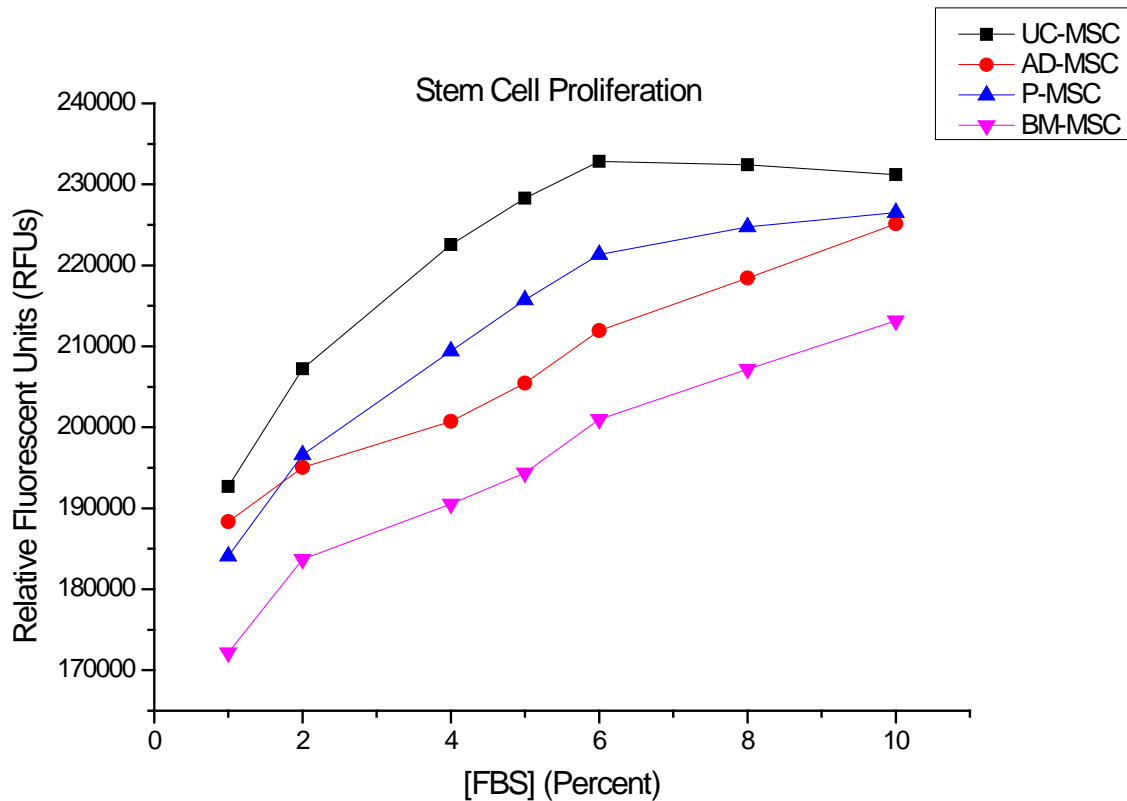UC-MSC had a maximum effect of FBS at 6%, while no saturation was seen in the other cell lines.

Figure 5. Proliferation of AD-MSCs, P-MSCs, BM-MSCs and UC-MSCs is shown in varying levels of FBS added to a serum-free base. RFU's at day 3 minus day 1 following Presto Blue exposure are shown as a function of [FBS]

Comparison of Differentiation Capacity

We also compared functional differentiation of AD-MSCs, P-MSCs, BM-MSCs and UC-MSCs. First, we determined tri-lineage differentiation into adipocytes, chondrocytes and osteoblasts. We used standard methods that showed equivalent differentiation between the MSCs derived from adipose, placental, bone marrow and umbilical tissues (data not shown). We also investigated differentiation into neural stem cells and the results of IHC marker expression are shown in Table 2. The markers Nestin, 3PDGH, GLAST, β3-Tubulin, MAP2 & Neurofilament M are specific to neural stem cells (Wu, R, et al, Cell Biol Int 2013; 37: 812) and while the various MSCs tested were positive for most markers, the P-MSCs and AD-MSCs were negative for GLAST while this antigen was expressed on cells derived from UC-MSCs as well as the control NSCs (hNSC). This suggests a difference in differentiation capacity in that UC-MSCs can fully differentiate into the NSC phenotype while AD-MSCs and P-MSCs do not using our differentiation protocol. This does not necessarily indicate a lack of capacity of P-MSCs or AD-MSCs to differentiate into NSCs.

	Nestin	3PDGH	GLAST	βIII-Tubulin	MAP2	Neurofilament M
AD-MSC	Positive	Positive	Negative	Positive	Positive	Positive
P-MSC	Positive	Positive	Negative	Positive	Positive	Positive
UC-MSC	Positive	Positive	Positive	Positive	Positive	Positive
hNSC	Positive	Positive	Positive	Positive	Positive	Positive

Table 2: Summary of IHC results as described in the Materials and Methods for the phenotypic identity of NSCs. While the control neural stem cells and those derived from UC-MSCs were positive for the essential markers of NSCs, the AD-MSC and P-MSC-derived cells were negative for GLAST.
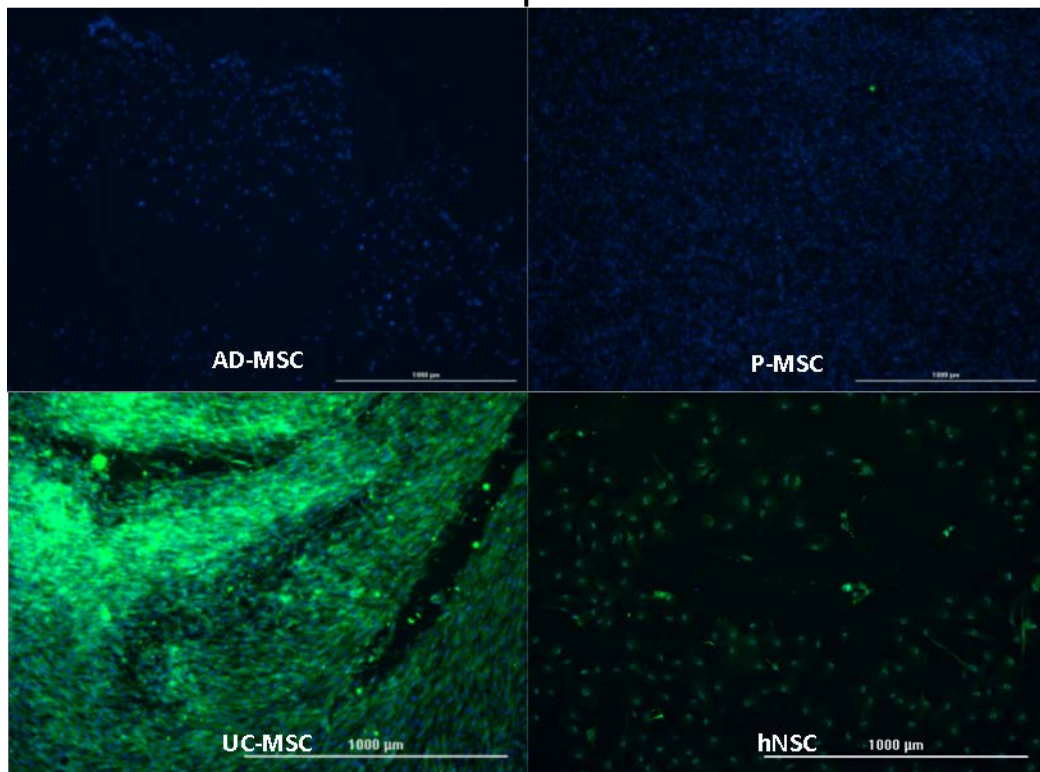

Figure 6 shows the IHC results of the staining for GLAST in P-MSCs, AD-MSCs, UC-MSCs and the control NSCs. While the cells differentiated from the UC-MSCs and the control NSCs were positive for GLAST, the AD-MSCs and P-MSCs were not. (The apparent difference between the UC-MSC-derived cells and and the control NSCs is due to cell density, both are positive for GLAST.)

Discussion

In the present study, we compared the cellular phenotype, potency, and functionality of expanded MSCs from different sources. Expanded MSCs were derived from lipoaspirate, bone marrow, placental decidua basalis, and Wharton's jelly of the umbilical cord. Our results showed expanded MSCs share universal properties, such as morphology, plastic adherence, and multi-lineage differentiation potential. We found variations between AD-MSCs, BM-MSCs, P-MSCs, and UC-MSCs in terms of growth rate, phenotypic characterization, potency, and functionality measurements.

We used quantitative assays to determine cell counts, viability, phenotype, potency by immuno-modulatory and mitochondrial function, and functionality by migration and proliferation. Variability in measurement was minimized by careful adherence to standard procedures including processing, analysis, and expansion. Additionally, each assay was performed at the same passage to avoid variation due to differences in passage number (Javazon EH, et al, Exp Hematol. 2004; 32:414).

International criteria of MSC identity was determined by flow cytometry according to ISCT standards (Dominici M, et al. Cytotherapy. 2006; 8: 315). Placental MSCs and bone-marrow MSCs did not achieve ISCT criterion values of CD_{45} and CD_{79a}. The increased expression of CD_{45} in P-MSCs may be due to associated leukocytes and CD_{79a} from residual B-cells.

Cellular potency is an important assessment of stem cells for clinical applications. We used quantitative assessment of mitochondrial function and immunosuppression as measures of cellular potency. Since MSCs are intrinsically immunosuppressive in nature, they can support graft survival and other clinical effects based on immunosuppression (Liu, R, et al., Stem Cells Dev 2013; 22:1053; Wang, LT, et al, J Biomed Sci 2016; 23: 76). However, the failure of MSCs to elicit immunosuppression is likely due to immune enhancing effects of MSCs triggered by proinflammatory cytokines, educed NO, etc while IDO expression induces immunosuppressive effects of MSCs. IDO has been proposed as a molecular switch to induce immunosuppression in MSCs (Li, W et al, Cell Death & Differentiation 19: 1505, 2012). We thus determined cellular potency by quantitation of γ-IFN induced IDO activity. The results showed maximum immunomodulatory potency in UC-MSCs, which was significantly greater than MSCs sourced from other tissues (Figure 2). This compares with other studies. Wang, Q, et al, (Human Vaccin & Immunother 12: 85, 2016) compared fetal BM derived MSCs, AD-MSCs and MSCs derived from Wharton's jelly of the umbilical cord. They found comparable phenotype, proliferation, clonality and that γ-IFN induced IDO expression was greatest in UC-MSCs, supporting our findings as well. Kim, JH, et al, (Stem Cells International, 2018: 8429042), also showed superior immunosuppression and minimal HLA-DR expression in UC-MSCs compared to AD-MSCs and periodontal ligament-derived MSCs Other reports of the comparison of MSC from various tissue sources also support biological advantages of UC-MSCs (Riordan, NH et al, J Transl Med 2018;16: 57; Najar, M, et al, Cell Immunol. 2010; 264:171; Weiss, ML, et al, Stem Cells 2006; 24: 781; Arutyunyan, I, et al, Stem Cell International 2016; 2016: 6901286) Jin, HJ, et al, (Int. J. Mol Sci 2013; 14: 17986), showed superior proliferation and anti-inflammatory properties of UC-MSCs compared to BM-MSCs and AD-MSCs.

Expanded MSCs showed measurable levels of cell-specific ATP content. However, cell-specific ATP expression was significantly higher in UC-MSCs supporting the assertion that they are the most potent type of MSC. Other studies have shown that ATP expression correlate with therapeutic outcomes in the transplantation of hematopoietic stem cells (Deskins D, et al, Stem Cells Transl Med. 2013; 2:151; Rich, IN Stem Cell Transl Med 2015; 4: 967).

Numerous clinical trials have been conducted and are presently ongoing for various MSC preparations (Carralho E, et al. Regen. Med. 2015; 10:1025; Freitag J, et al. BMC Musculoskeletal Disorders 2016; 17: 230; Neirinckx V, et al. Stem Cell Trans. Med 2013; 2: 284; Munir H and McGettrick, HM Stem Cells Dev. 2015; 24:2091). From the results reported here it would be expected that that expanded UC-MSCs exhibit greater therapeutic benefit than other impure sources of MSCs such as bone marrow aspirate and stromal vascular fraction. Direct clinical comparisons from various sourced MSCs are lacking. Mechanisms of stem cell therapy include paracrine effects from stem cell-derived biological factors eliciting anti-inflammatory & neural protective effects, differentiation of stem cells into other cellular lineages, and intercellular communication through tunneling nanotubes.

Conclusion

Our results show bio-similarity between stem cells derived from adipose, bone marrow, placental and umbilical cord tissues regarding expansion, trilineage differentiation, and phenotypic characterization by flow cytometry according to the ISCT definition of MSCs. While all sources of MSCs also exhibited activity in potency assays including quantitative assessment of mitochondrial function and immunosuppression, cell migration and proliferation, there were clear differences. Our results revealed significant superiority of UC-derived MSCs as was also found in similar studies performed in several other laboratories. Age of the cells may be a factor in the overall performance of MSCs. Furthermore, the capacity to differentiate into neural stem cells varied between MSC derived from UC, adipose and placental tissues with UC derived MSCs expressing all NSC markers while adipose and placental-derived MSCs did not express GLAST under identical conditions. Thus, while MSCs from various tissues show similarity, there are also multiple characteristics of umbilical cord MSCs significantly superior to those derived from adipose, bone marrow or placental tissues. This suggests that UC-MSCs may also exhibit superior therapeutic benefit.