Cytotechnology (2016) 68:2061–2073
DOI 10.1007/s10616-016-9946-5

ORIGINAL ARTICLE

Generation and characterization of human cardiac resident and non-resident mesenchymal stem cell

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Received: 2 October 2015 / Accepted: 14 January 2016 / Published online: 28 January 2016

Abstract Despite the surgical and other insertional interventions, the complete recuperation of myocardial disorders is still elusive due to the insufficiency of functioning myocardiocytes. Thus, the use of stem cells to regenerate the affected region of heart becomes a prime important. In line with this human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) have gained considerable interest due to their potential use for mesodermal cell based replacement therapy and tissue engineering. Since MSCs are harvested from various organs and anatomical locations of same organism, thus the cardiac regenerative potential of human cardiac-derived MSCs (hC-MSCs) and human umbilical cord Wharton's Jelly derived MSC (hUC-MSCs) were tested concurrently. At in vitro culture, both hUC-MSCs and hC-MSCs assumed spindle shape morphology with expression of typical MSC markers namely CD105, CD73, CD90 and CD44. Although, hUC-MSCs and hC-MSCs are identical in term of morphology and

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immunophenotype, yet hUC-MSCs harbored a higher cell growth as compared to the hC-MSCs. The inherent cardiac regenerative potential of both cells were further investigated with mRNA expression of ion channels. The RT-PCR results demonstrated that both MSCs were expressing a notable level of delayed rectifier-like K^+ current (I_{KDR}) ion channel, yet the relative expression level was considerably varied between hUC-MSCs and hC-MSCs that Kv1.1(39 ± 0.6 vs 31 ± 0.8), Kv2.1 (6 ± 0.2 vs 21 ± 0.12), Kv1.5 (7.4 ± 0.1 vs 6.8 ± 0.06) and Kv7.3 (27 ± 0.8 vs 13.8 ± 0.6). Similarly, the $Ca2^+$-activated K^+ current (I_{KCa}) channel encoding gene, transient outward K^+ current (I_{to}) and TTX-sensitive transient inward sodium current ($I_{Na.TTX}$) encoding gene (Kv4.2, Kv4.3 and hNE-Na) expressions were detected in both groups as well. Despite the morphological and phenotypical similarity, the present study also confirms the existence of multiple functional ion channel currents I_{KDR}, I_{KCa}, I_{to}, and $I_{Na.TTX}$ in undifferentiated hUC-MSCs as of hC-MSCs. Thus, the hUC-MSCs can be exploited as a potential candidate for future cardiac regeneration.

Keywords Mesenchymal stem cell · Electrophysiology · Cardiac resident stem cell and umbilical cord stem cell

Abbreviations

MSCs	Mesenchymal stem cells
BM-MSCs	Bone marrow-derived MSCs
CSC	Cardiac stem cells
hC-MSC	Human cardiac MSC
hUC-MSCs	Human umbilical cord derived MSCs

Introduction

Despite considerable clinical advances over the past few decades, cardiovascular disease remains a leading cause of death worldwide. The massive loss of functional cardiomyocytes leads to irreversible myocardial anomalies. Unlike highly repopulating organs, cardiomyocytes possesses a limited capacity for the regeneration especially in conditions like coronary arterial disease and acute myocardial infarction (AMI), which still represents a significant cause of mortality and morbidity worldwide (Gallina et al. 2015).

Over the years, stem cell based therapies have become an 'elixir' in treating many human diseases. Enormous research in stem cell biology has opened a new paradigm in understanding the pathology and therapeutic approach of particular disease or system. In most of the studies with disclosed trial outcomes, a desired output were observed as the administration of MSCs considerably enhanced the final cardiac function. However, the subjected reparative mechanisms that paved such improvement were not fully elucidated. Among all tested sources, including bone marrow, cardiac biopsy and perinatal tissues, human umbilical cord derived-MSCs stand as a champion candidate due to an unlimited availability of the samples, rapid in vitro expansion and lesser ethical issues. In regard to this, among newly registered trials on MSCs and myocardial infarction, a substantial number of studies are consuming hUC-MSC to further explore the efficiency of MSC in recuperate myocardial infarction-mediated injuries (https://clinicaltrials.gov/ct2/results?term=mesenchymal+stem+cells+AND+myocardial+infarction).

Traditionally, adult cardiomyocytes are believed to be terminally differentiated mature cells with no regenerative capacity, however, the current findings are slightly against this concept with discovery of cardiac stem cell (CSC, c-kit+) where CSC are considered as cardiac resident stem cells (Kazakov et al. 2015). Hence, the identification of a specific cell population within the myocardium that are capable of differentiating into cardiomyocytes, known as resident cardiac stem cells (CSC) has resuscitated the treatment options for degenerative myocardial diseases. The endogenous (resident) cardiac stem cells or progenitor cells (c-kit+ CSCs) that are derived from autologous/syngeneic heart tissue biopsies are being explored as a reliable source for treating myocardial infarction (Fuentes et al. 2013). The tissue resident cardiac stem cells (CSC) were shown to form all three cardiac lineages such as cardiomyocytes, smooth muscle cells and endothelial cells at in vitro environment (Fuentes et al. 2013). Despite the parenchymal stem cells of CSC, a stem cell that forms a mesenchymal scaffold to consolidate the organ which is termed as mesenchymal stem cells (MSCs) also were reported to be present in human heart tissue. Although there is no clear difference or boundary between CSC and hC-MSC, yet the physiological contribution hC-MSCs at pathological circumstances in regenerating an exhausted

cardiac damage and controlling excessive immune response could not be negated. Indeed, hC-MSCs are also believed to be an originator for cardiac stem/progenitor cells that carries the common features of mesodermal lineage. Although, hC-MSCs are an ideal for myocardial regeneration due to their reliable and targeted differentiation towards various cardiac cells, however, the rare frequency of CSC and the highly invasive procedure for harvesting impeded the potential use in therapeutic or research applications. The physiological role of CSC in recuperating or regenerating the infarcted cardiac region appears minimal and functionally insufficient (Weil and Canty 2013). Therefore, implantation of ex vivo expanded cells with cardiac regenerative potential may provide a meaningful replacement for the damaged cardiac cells. The highly demanding technical feasibility and bio-availability of resident cardiac stem cells hamper their potential use in clinical applications, thus necessitate a desperate need to find an alternative source of stem cell for cardiac regeneration.

Considering the acquired myocardial disorders are form of degenerative diseases that often involve an excessive inflammation, thus MSC from other organs could serve as an ideal replacement for the hC-MSC (Mazhari et al. 2007; Heubach et al. 2004; Caplan 2007). Beside the targeted differentiation, MSCs also migrate and home at the injured/inflamed tissues to expedite the tissue repair by secreting growth factors and harnessing the uncontrolled immune response (Kinnaird et al. 2004; Pereira et al. 2008; Ramasamy et al. 2008). Under a specific inductive condition, MSCs are capable of differentiating into mesodermal lineage cells, including cardiomyocytes (Sanchez et al. 2000; Deans and Moseley 2000; Forbes et al. 2002).

Initially, bone marrow was considered as a major source of MSCs and was widely utilized to study at both experimental and clinical levels (Orlic et al. 2001; Li et al. 2005; Heubach et al. 2004). It has been showed that the bone marrow-derived MSCs (BM-MSCs) exhibit a promising therapeutic value in treating heart diseases (Miyahara et al. 2006; Obradovic et al. 2004). Unfortunately, the quantity and qualities (proliferation efficiency and differentiation potential) of BM-MSCs are found to be significantly declined with aging that necessitated the search for an alternate source of MSCs (Rao and Mattson 2001; Mueller and Glowacki 2001). Almost all organs contain MSC as many studies reported the successful generation of MSCs from human skin, liver, heart, umbilical cord and cord blood (Hoogduijn et al. 2007; Goodwin et al. 2001; Feldmann et al. 2005; Bieback et al. 2004; Mc- Elreavey et al. 1991; Wang 2004). Amongst, hUC-MSCs stand out a champion candidate due to unlimited availability of the samples, rapid in vitro expansion and lesser ethical issues (Wang et al. 2004; Weiss et al. 2006). Numerous pre-clinical studies had demonstrated that hUC-MSCs are able to induce cardiac regeneration via various mechanisms ranging from a simple tropic cytokine secretion to the direct differentiation into cardiomyocyte when implanted/infused to the animal myocardial infarction models (Santos et al. 2014; Miyahara et al. 2006).

Although the surface antigen profiles and differentiation potential of hUC-MSC and hC-MSC are well documented, the electrophysiological properties of ion channels in undifferentiated hUC-MSCs and hC-MSC are still elusive (Shake et al. 2002; Pereira et al. 2008; He et al. 2011; Bearzi et al. 2009). Ion channels (delayed rectifier-like K^+ current (I_{KDR}), $Ca2^+$-activated K^+ current (I_{KCa}), transient outward K^+ current (I_{to}) and TTX-sensitive transient inward sodium current ($I_{Na.fTTX}$) are widely expressed in different types of cells to regulate the physio-biochemical reactions as they play a fundamental role in maintaining the physiological homeostasis, cell proliferation, and signal transduction at different cell stages (Bai et al. 2007; Chen et al. 2007; Nilius and Droogmans 2001). Unlike other mature cells, cardiac cells are rigidly controlled cells that produce and conduct impulse to mediate synchronized contraction for uninterrupted myocardial function. Thus, transplantation of ill-differentiated stem cells or inappropriate stem cells that are incompetent in signal transduction might jeopardize the physiological function of the heart. The therapeutic use of MSCs to induce myocardial regeneration is considered as safe. To date, there have been no reports of cardiac arrhythmia in patients who had received adult bone marrow MSCs for cardiac diseases. However, in certain conditions, cardiomyocytes derived from other pluripotent stem cells namely human embryonic stem cells had potentiated the arrhythmic action (Zhang et al. 2002).

Therefore, it is an utmost importance to decipher the inherent nature of MSCs in conducting

electrophysiological signals through various ion channels. Currently there are only few reports available on the electrophysiological properties of cardiac resident and non-resident MSCs (Bai et al. 2007; Chen et al. 2007; Nilius and Droogmans 2001). Hence, the present study was designed to investigate the electrophysiological properties of human umbilical cord-derived mesenchymal stem cell as a non-resident stem cell and compared with the resident stem cell of cardiac-derived mesenchymal stem cells.

Materials and methods

Generation and culture of hUC-MSCs

The present study was conducted after written informed consent obtained from parents in accordance with the ethical committee requirements of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The isolation and culture of hUC-MSC was carried out by the method as described previously (Tong et al. 2011). Umbilical cord tissues were minced into smaller pieces and enzymatically digested with 0.4 % type II collagenase (Worthington, Lakewood, NJ, USA) and 0.01 % DNAse (Worthington, Lakewood, NJ, USA) followed by mechanical dissociation using a hand held cell homogenizer (Hassen Wagger) at 9000 rpm for 10–15 min. The cell suspension was filtered using 70 µm cell strainer and washed. The cell pellet was re-suspended and cultured in MSC complete medium that contained Dulbecco's Modified Eagle's medium with nutrient mixtures F-12 (HAM) (1:1), GLUTAMAX-IX (200 mM L-alanyl-L-glutamine dipeptide) (Gibco, Invitrogen, Carlsbad, CA, USA), 10 % fetal bovine serum (Stem Cell Technology Inc., London, UK), 1 % Penicillin and Streptomycin (Gibco, Invitrogen), and 0.1 % Gentamicin (Gibco, Invitrogen). The single cells were seeded in T25 culture flask (Falcon, Tissue Culture Treated Flask) at 30×10^6 cells in 5 ml of complete medium as described previously (Vellasamy et al. 2012). Cells were incubated at 37 °C in humidified 5 % CO_2 incubator and non-adherent cells were removed by subsequent medium change. Upon 70–80 % confluency, the adherent cells were harvested (passage-0) by trypsinisation process (0.25 % trypsin–EDTA, Invitrogen, BRL, Burlington, ON, Canada).

Generation and culture of hC-MSC

Myocardial biopsies were collected from the patients undergoing heart surgery after written, informed consent in accordance with the ethical committee requirements of National Heart Institute Malaysia. The generation and culture of hC-MSC was carried out by previously established method (Takehara et al. 2008). Briefly, myocardial biopsies collected from the patients were minced into small pieces, subjected to enzymatic digestion prior to culture in MSC complete medium in a 37 °C humidified 5 % CO_2 incubator for 7–10 days. Upon reaching 70–80 % confluence, adherent cells were harvested (passage-0) via trypsinization (0.25 % trypsin–EDTA, Invitrogen, BRL, Canada) for use in downstream experiments.

Cell passage

Early passages (P0–P3) of both hUC-MSC and hC-MSC at cell density of 5×10^3 cells/cm^2 were cultured using MSC complete medium and cultures were maintained at 37 °C in a humidified atmosphere containing 5 % CO_2, with a change of medium every 3–4 days. When cultures reached 80–90 % of confluency, cells were detached by using 0.25 % trypsin–EDTA (Invitrogen, BRL, Canada), either consumed for downstream experiments or sub-cultured further till passage 15.

Immunophenotyping of hUC-MSC and hC-MSC

Expression of putative surface proteins was assessed by flow cytometer analysis (Mindaye et al. 2015). The following monoclonal antibodies that have been raised in mouse and were specific for human antigens, namely CD73-PE (mouse anti-human), CD90-FITC (mouse anti-human), CD44-PE (Mouse Anti-human) and CD105-PerCP-Cy (mouse anti-human) (BD Biosciences, San Jose, CA, USA) were used to label MSCs as per manufacture's instruction. Stained cells were re-suspended in PBS and analyzed by using flow cytometry (FC- 500 Beckman Coulter, Brea, CA, USA). The acquired data were analyzed using CXP software provided by the manufacturer.

Growth kinetics and doubling time by MTT assay

The hUC-MSC and hC-MSC (1×10^3 cell/well) were plated in 96-well plates every day for 5 days and

incubated at 37 °C in a 5 % CO_2 humidified incubator. Medium was changed once in 2 days and triplicates of hUC-MSC and hC-MSC were maintained. On day 6, MTT (3-(4, 5-dimethylthiazol- 2, 5–diphenyl tetrazolium bromide) (Sigma Chemical Co., St. Louis, MO, USA) proliferation assay was carried out to assess the viability and proliferation rate of hUC-MSC and hC-MSC. MTT was dissolved in PBS at 5 mg/ml; added to the culture medium at a dilution 1:10 prior to be incubated at 37 °C for 4 h. The medium was aspirated; 100 µl of DMSO was added/well and the 96-well plate was read using a microplate reader (DYNEX Technologies, Chantilly, VA, USA) at the wavelength of 570 nm.

Growth kinetics and doubling time by Trypan blue assay

The hUC-MSC and hC-MSC (1×10^3 cell/well) were plated in 96-well plates for 5 days and incubated at 37 °C in a 5 % CO_2 humidified incubator. Medium was changed once in 2 days and triplicates of hUC-MSC and hC-MSC were harvested every day for 5 days using 0.25 % trypsin–EDTA.The growth curve of hUC-MSC and hC-MSC were determined manually by performing cell counting using trypan blue exclusion method. The initial seeding, cell yields and days in culture were recorded; the doubling time was determined using the Patterson Formula [Td = Tlg2/lg (Nt/N0)], Td is the doubling time (h), T is the time taken for cells to proliferate from N0 to Nt (hour), and N is the cell count.

Differentiation assay

The hUC-MSCs and hC-MSCs were grown till reaching 90 % of cell confluency in MSC complete medium and then the cells were induced to differentiate into adipocytes and osteocytes by supplementing the respective differentiation medium (STEMPRO®). After differentiation, the adipocytes and osteocytes were fixed with 4 % formalin and stained with Oil Red O Solution and Alizarin Red respectively.

Semi quantitative RT-PCR

Total RNA was extracted from hC-MSC and hC-MSC by using RNeasy Mini Kit (QIAGEN). The preparation of the first-strand cDNA was conducted following the instruction of the QuantiTect (Qiagen). RT-PCR was performed using the QuantiTectRev.Transcription Kit (QIAGEN, Valencia, CA, USA). Genes of interest were obtained using primers synthesized from Xcelris Labs Limited (Gujarat, India) as shown in Table 1. Gene expression was quantified by ImageJ software and the gene expression was normalized with housekeeping gene, GAPDH and ß-Actin.

Statistical analysis

Data from each group were expressed as mean and standard error (SE) of at least three separate experiments performed. Statistical comparison between groups was analyzed using Student's t test. A value of $^*P < 0.05$ was considered to be statistically significant.

Results

Characterization of MSCs derived from human umbilical cord and heart tissue

At in vitro culture, adherent cells from human umbilical cord and cardiac tissues acquired a homogeneous population at passage 3, hence the morphological comparison was conducted from passage 4 onward. Microscopical observation showed that the morphological appearance of both hUC-MSCs and hC-MSCs was comparable as both MSCs exhibited a fibroblast like appearance or spindle-shaped morphology (Fig. 1a, b). However, upon passage 10, hC-MSCs had gradually lost their original morphology and acquired a more flatten and polygonal morphology indicative of cellular senescence. On the other hand, hUC-MSCs retained a stable morphology until passage 15 (data not shown). Immunophenotyping was conducted to further characterize MSCs based on cell surface protein expression via flow cytometer at passage 4. Flow cytometry analysis revealed that both hUC-MSCs and hC-MSCs cells positively expressed (>95 %) the putative mesenchymal stem markers namely CD44, CD73, CD90 and CD105 (Fig. 1c, d). The average expression for hUC-MSC (shown in table Fig. 1e) was: CD44 (99.9 ± 0.0 %), CD73 (100 ± 0.0 %), CD90 (99.9 ± 0.1 %) and CD105 (99.6 ± 0.2 %). Similarly, hC-MSC expressed positive surface markers which accounted for CD44 (98.8 ± 1.0 %), CD73 (99.7 ± 0.1 %), CD90 (99.9 ± −0.1 %), and CD105 (96.5 ± 1.5 %).

Table 1 List of primers and annealing temperature used for RT-PCR

Gene	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)	bp	T_{ane}
Kv1.1	CCATCATTCCTTATTTCATCAC	CTCTTCCCCCTCAGTTTCTC	488	51
Kv1.5	TGCGTCATCTGGTTCACCTTCG	TGTTCAGCAAGCCTCCCATTCC	906	52
Kv2.1	TACTGGGGCATCGACGAGA	GACTGGCCGAACTCATCGA	308	53
Kv4.2	ATCTTCCGCCACATCCTGAA	GATCCGCACGGCACTGTTTC	362	49
Kv4.3	GATGAGCAGATGTTTGAGCAG	AGCAGGTGGTAGTGAGGCC	106	49
Kv7.3	GGAGAGGAGATGAAAGAGGAG	TGAAGAAAGGAAAAGAGACGAC	358	57
KCNN3	GCCATCCTCCACCCTTCCTCCA	CGGGAGGAGATGACGATCTC	320	57
KCNN4	CCTTTCAGACACACTTTGGCTGATCC	CAGTGCTAAGCAGCTCAGTCAGG	529	60
hNE-Na	GCTCCGAGTCTTCAAGTTGG	GGTTGTTTGCATCAGGGTCT	446	59
GAPDH	TCCCTGAGCTGAACGGGAAG	GGAGGAGTGGGTGTCGCTGT	217	52
β-actin	CACGAAACTACCTTCAACTCC	CATACTCCTGCTTGCTGATC	265	56

Growth kinetics of hUC-MSCs and hC-MSCs

The growth kinetics and cell proliferation fold changes of hUC-MSCs and hC-MSCs were plotted at passage 4. The manual cell counting by trypan blue exclusion method revealed that hUC-MSCs remained in lag phase up to 48 h and assumed a rapid acceleration log phase till 96 h. Although, hUC-MSCs were projected to achieve the plateau phase at 120 h, yet an extraordinary rise in cell proliferation was noticed between 96 and 120 h. In contrast, hC-MSCs entered into log phase after 48 h; steadily accelerated into log phase until 96 h and accomplished a plateau phase after 96 h (Fig. 2A, B). The growth kinetics of these cells were further confirmed and compared using MTT assay at different time points, too. In term of robustness of proliferation, hUC-MSCs achieved the double fold change of proliferation between 48 and 72 h whereas hC-MSCs had doubled the cell numbers approximately at 72 h. This result further confirms that hUC-MSC has shorter doubling time than hC-MSC (Fig. 2C, D).

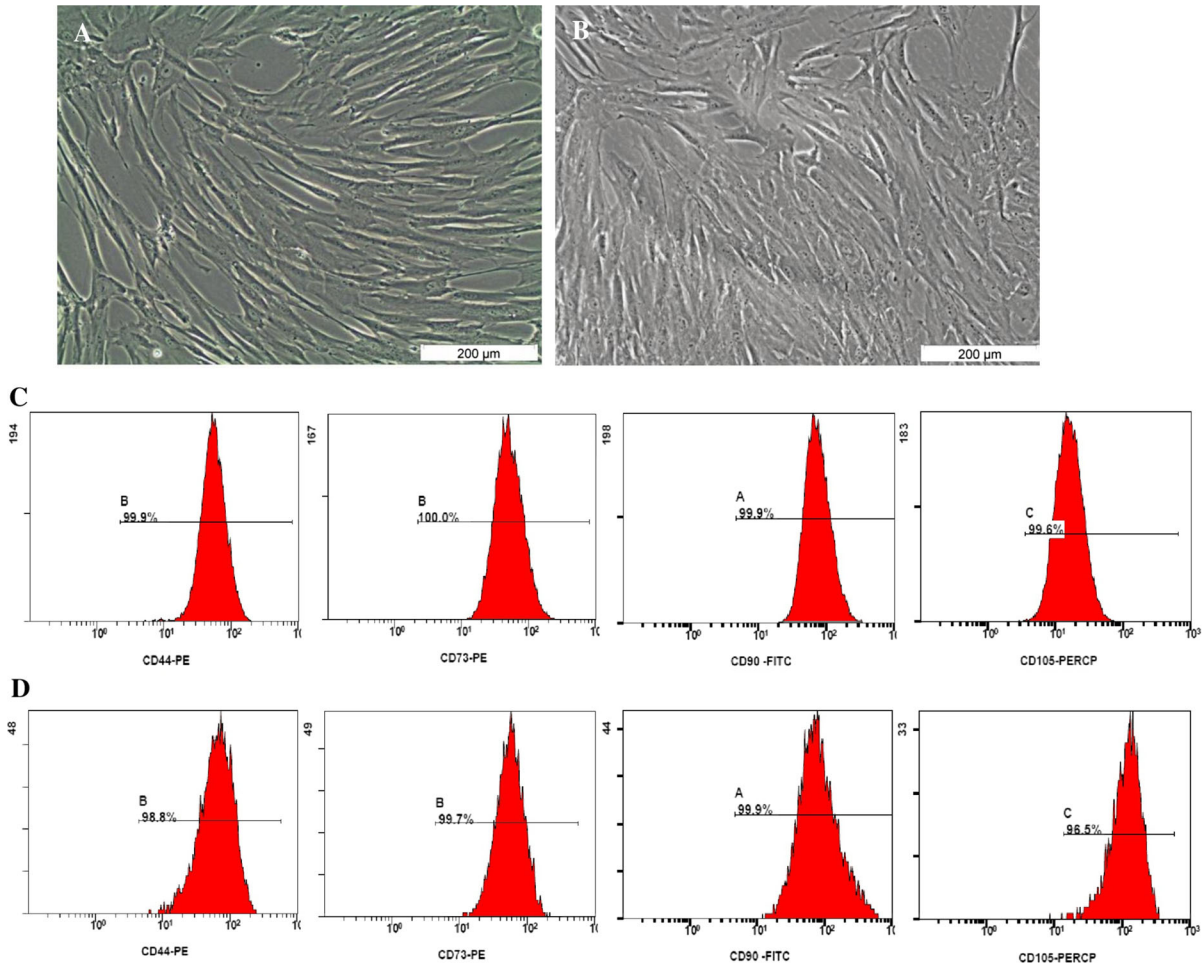
Differentiation potential of hUC-MSCs and hC-MSCs

To evaluate the mesodermal lineage differentiation ability of hUC-MSCs and hC-MSCs, cells were cultured in respective adipogenic and osteogenic induction media along with complete MSC culture medium as a control. Microscopical observation of both hUC-MSCs and hC-MSCs in adipogenic induction medium exhibited occurrence of numerous intracellular bright white oil droplets. Further histochemical evaluation with Red O oil stain showed red vesicles that are indicative of fat droplets in the cytoplasm. In contrast, no lipid droplets or auto differentiation were observed in MSCs cultured in control medium (Fig. 3a). Whereas, osteogenic induction resulted in deposition of calcium minerals (calcification) was assessed by Alizarin Red stain. Figure 3b showed that red staining of mineralized calcium deposition was observed in both MSC groups whereas such calcium deposition was not observed in cells cultured in MSC culture medium.

Ion channel gene expression in hUC-MSCs and hC-MSCs

The expression of ion channel subunits was observed in undifferentiated hUC-MSC as well as hC-MSC. Semi quantitative RT-PCR results provide a molecular basis for the functional ionic currents (I_{KDR}, I_{KCa}, Ito and $I_{Na.TTX}$) in both hUC-MSC and hC-MSC. Positive mRNA expression of Kv1.1, Kv2.1 and Kv1.5 indicated that these ion channel subunits are responsible for delayed rectifier K^+ current (I_{KDR}). The expression of KCNN3 and KCNN4 specifies the presence of $Ca_2{}^+$-activated K^+ current of I_{KCa}. Expression of Kv4.2 and Kv4.3 is responsible for transient outward K^+ current (Ito) and the expression of hNE-Na for the presence of $I_{Na.TTX}$ (Fig. 4A).

The relative expression of ion channels was estimated and compared between hUC-MSC and hC-MSC. Human heart tissue was used as a positive control. Since, the expression level of delayed rectifier-like K^+ current (I_{KDR}) ion channels was found to



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	Surface expression (%)			
Type of cell	CD44	CD73	CD90	CD105
huUC-MSC	99.9± 0.0	100± 0.0	99.9± 0.1	99.6± 0.2
huC-MSC	98.8± 1.0	99.7± 0.1	99.9± 0.1	96.5± 1.5

Fig. 1 Characterization of mesenchymal stem cells (MSC) derived from human umbilical cord and heart biopsy. **a**, **b** Images are showing morphology of adherent hUC-MSC and hC-MSC. **c** Flow Cytometry analysis showing the immunophenotype of hUC-MSC and hC-MSC. *Upper panel* histogram shows the expression of positive markers for hUC-MSC. **d** *Lower panel* histogram shows the expression of positive markers for hC-MSC. **e** The *table* shows the mean value of percentage of positive cells (±) standard deviation to the total number of sample analyzed (n = 3). Cells used in this analysis were obtained from the homogenous confluent monolayer at the end of third/fourth passage. The picture was taken using phase contrast microscope at 100× magnification. *Scale bar* = 200 µm

be present in both groups. However, the relative expression level was considerably different between hUC-MSC and hC-MSC: that Kv1.1 (39 ± 0.6 vs 31 ± 0.8), Kv2.1 (6 ± 0.2 vs 21 ± 0.12), Kv1.5 (7.4 ± 0.1 vs 6.8 ± 0.06) and Kv7.3 (27 ± 0.8 vs 13.8 ± 0.6). Similarly, the Ca^{2+}-activated K^+ current (I_{KCa}) channel encoding gene expression was detected in both groups and the relative expression level was significantly increased in hC-MSC when compared to hUC-MSC as for KCNN3 (34 ± 0.05 vs 54.7 ± 0.13)

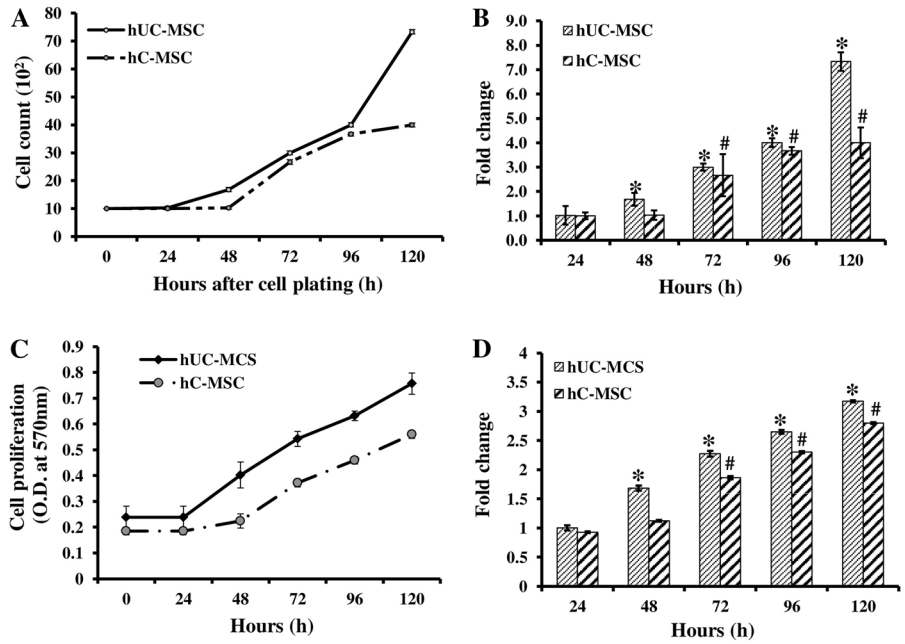

Fig. 2 Proliferation rate and fold change of hUC-MSCs and hC-MSCs. **A** 1×10^3 cells/well of hUC-MSCs and hC-MSCs were seeded into 96 well plates and harvested by Trypan blue dye exclusion test after 24, 48, 72, 96 and 120 h incubation by trypsinization for Trypan blue dye exclusion test after 24, 48, 72, 96 and 120 h incubation. **B** The fold change was checked over a period of 120 h. **C** 1×10^3 cells/well hUC-MSC and hC-MSCs were seeded into 96 well plates and incubated over 24, 48, 72, 96 and 120 h. The MTT reagent was added post incubation at each time points and measured with ELISA plate reader. Cell proliferation was plotted using O.D absorbance values. **D** The fold change was calculated over a period of 120 h. The variation within each set of triplicates is shown with mean ± SD: $^{*\#}$ $P < 0.05$ (n = 3)

and KCNN4 (4 ± 0.6 vs 14 ± 0.3). The other two types of channels: transient outward K^+ current (I_{to}) and TTX-sensitive transient inward sodium current ($I_{Na.TTX}$) encoding gene (Kv4.2, Kv4.3 and hNE-Na) expression level was comparable between both groups. Collectively, the result suggest that the gene expression pattern of ion channel currents I_{KDR}, I_{KCa}, I_{to}, and $I_{Na.TTX}$ was considerably different between the groups.

Ion channel gene expression between cardiomyocyte and mesenchymal stem cells

The heart biopsy has heterogeneous cell population which is called committed progenitor cells such as cardiac progenitor cells. The progenitor cells may affect the expression of ion channels. In addition, ion channel expression may change with cell cycle progression (Pardo et al. 1998) but can also vary with different progenitor lineages and stages of our cell population in vitro. Therefore the expression of mRNA in each type of cells was compared against heart biopsy cells (Fig. 4B).

The delayed rectifier-like K^+ current ion channel subtype of Kv1.1 expression level in human heart tissue was close to that of hUC-MSC (39 ± 0.6 vs 36.2 ± 0.3), but it was significantly different from hC-MSC (31.5 ± 0.8), whereas, mRNA expression of ion channel subtype of Kv2.1in human heart tissue was comparably higher (46.7 ± 0.2) than for hC-MSC (21 ± 0.1) and hUC-MSC (6 ± 0.2). Similarly, the expression level of Kv7.3 in human heart tissue was significantly stronger (31.8 ± 0.2) than for hC-MSC (13.8 ± 0.6) and hUC-MSC (27.3 ± 0.8). However, no significant variation was observed in Kv1.5 expression by hC-MSC and hUC-MSC when compared to human heart tissue. The second type of ion channel (I_{KCa}) subunit, KCNN3 mRNA expression was found to be significantly higher in heart biopsy (63.78 ± 0.07) when compared to hUC-MSC (34.42 ± 0.6) and hC-MSC (54.80 ± 0.13). In contrast, the mRNA expression of KCNN4 ion channel subtype was more strongly expressed in hC-MSC

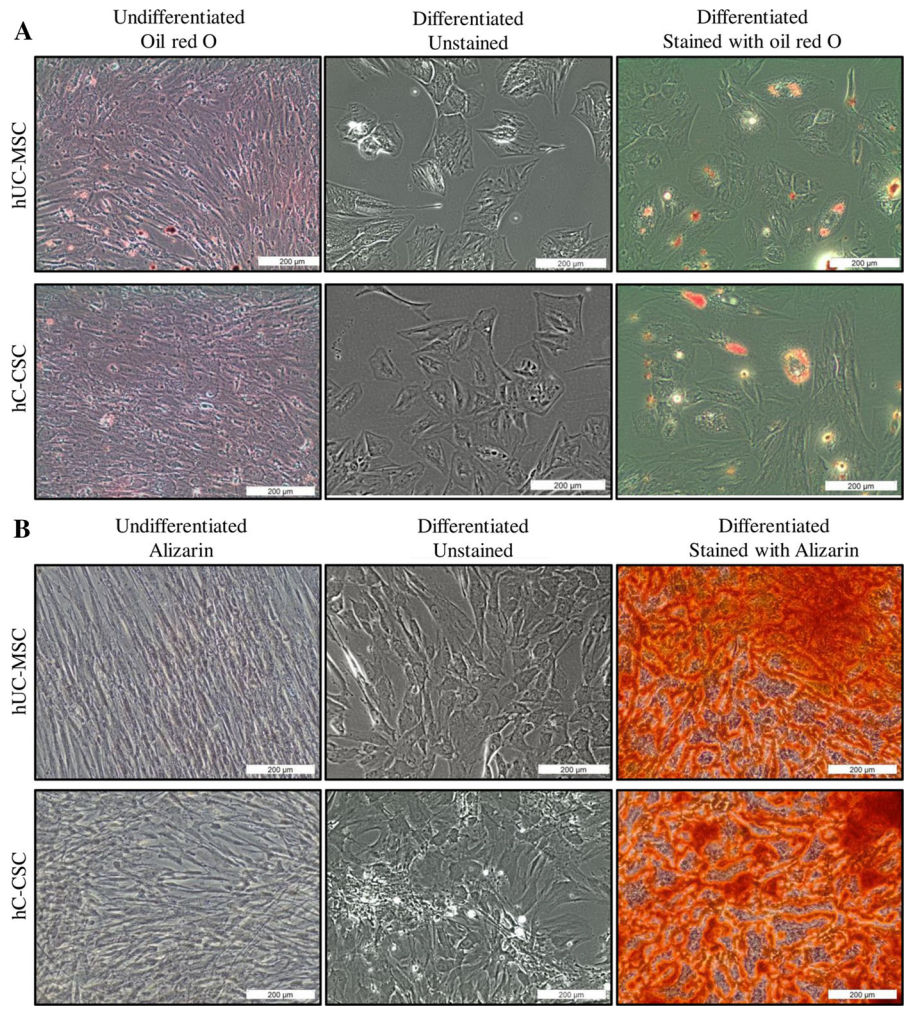

Fig. 3 Characterization of the two-lineage potential of mesenchymal stem cells (MSCs) from human umbilical cord (hUC) and heart biopsy at Passage 4. **a** Morphological appearance of undifferentiated and adipogenic differentiated MSC's. *Red* color stained cells indicating the accumulation of fat droplets in adipogeneic lineage cells, were not seen in undifferentiated MSC's. **b** Morphological images of undifferentiated and osteogenic differentiated MSC's. *Red* color stained cells indicate the presence of calcium mineralized droplets in osteogeneic lineage MSC's. The picture was taken using phase contrast microscope at 100× magnification. *Scale bar* = 200 μm. (Color figure online)

(14.4 ± 0.3) than in heart biopsy (3.39 ± 0.4) and in hUC-MSC (4.21 ± 0.7). Likewise, the other type of ion channel (Ito) subtype Kv4.2 was significantly down-regulated (11.4 ± 0.5) in heart biopsies when compared to hUC-MSC (35.5 ± 0.3), hC-MSC (40 ± 0.47), whereas the expression level of other subtype Kv4.3 was almost equal to heart biopsy. The fourth type of ion channel ($I_{Na.TTX}$) subunit hNE-Na was significantly down-regulated in human heart biopsies (12.2 ± 0.2) when compared hUC-MSC (27.7 ± 0.4) and hC-MSC (28.6 ± 0.33).

Discussion

In the present study, MSCs derived from human umbilical cord and human cardiac tissues were compared in term of morphological & growth analysis, immunophenotyping, mesodermal differentiation and ion channel gene expression under standard generation and expansion protocols. Based on the mesenchymal stem cell's characteristics delineated by the International Society for Cell Therapy (ISCT), MSCs derived from human umbilical cord and cardiac tissue were not similar in term of morphology, expression of surface markers, colony formation and the mesodermal differentiation ability. Both cells were adherent to the plastic surface and formed a fibroblast like spindle shape at early passages. However, upon expansion, hC-MSC gradually acquired a senescence-like phenotype beyond passage 5 but reconstituted to the spindle shape morphology upon basic fibroblast growth factor (bFGF) supplementation. In contrast, hUC-MSCs retained a fibroblastic morphology until

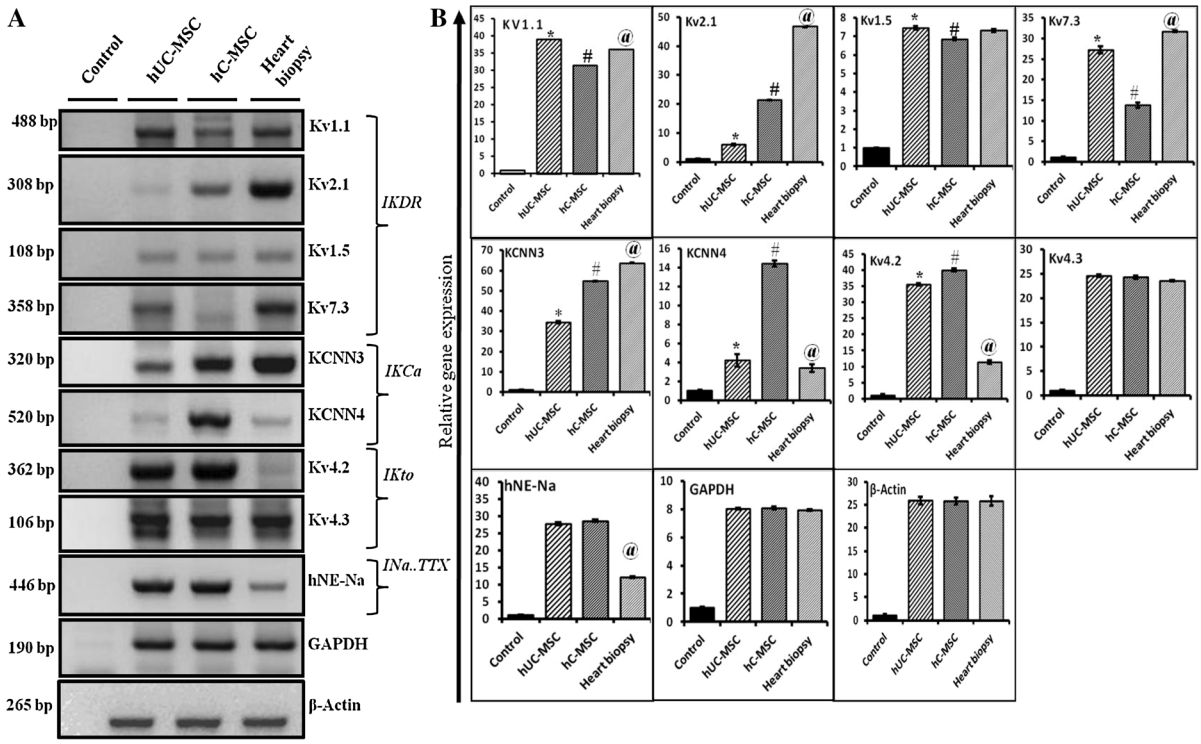

Fig. 4 Characterization of multiple functional ion channel currents subtypes in different sources of MSCs. **A** Reverse transcription-polymerase chain reaction data compared between hUC-MSCs, hC-MSCs and heart biopsy. The gel image on the *left* is showing the mRNAs expression of ion channel subunits. Primer and heart biopsy mRNA were used as a negative and positive control, respectively. GAPDH and ß-Actin were used as an internal control gene. The experiment was conducted in replicate of technical triplicates. **B** *Bar graphs* comparing the relative mRNA expression of functional ion channel currents (K^+ and Na^+) between experimental groups. The expression of K^+ channel current was analyzed by quantification of Kv1.1, Kv2.1, Kv1.5, Kv7.3, KCNN3, KCNN4, Kv4.2, Kv4.3 gene expressions and Na^+ channel current was hNE-Na gene expression. The sources of mRNAs of these cells were obtained from the homogenous confluent monolayer at fourth passage. The variation within each set of triplicates is shown with mean of SD $\pm$: $^{*\#@}P < 0.05$ (n = 3)

passage 10 of the culture (data not shown). Although both MSCs were efficiently amplified under a standard culture condition (Pereira et al. 2008; Hartmann et al. 2010; Chong et al. 2011), yet a prominent difference in term of doubling time and growth kinetics were noticed. Trypan blue and MTT assay clearly demonstrated that hUC-MSCs inherited a faster doubling time between 48 and 72 h than those of hC-MSC ($\sim$72 h). An extensive proliferative capability was noticed in hUC-MSC compared to hC-MSC. Amongst all other sources, hUC-MSCs are often considered as rapidly expanding cells. Cells from early human development such as pre and peri natal tissues-derived stem cells are known for rapid in vitro culture growth due to unshorten telomere length (Shaer et al. 2014).

The superior proliferative capacity of hUC-MSCs at in vitro culture is highly acceptable as hUC-MSCs are rather primitive cells with intact telomere length at early passage (Von Zglinicki and Martin-Ruiz 2005). However, further analysis is required to confirm whether the differences between hUC-MSCs and hC-MSCs are due to inherent, or aging (52 ± 6) of the heart sample. Even though, hUC-MSC and hC-MSC are derived from mesenchyme and categorized as adult multipotent stem cell, but the similarities of the surface antigen profiles and differentiation potential have not been well documented. Many subsequent studies have demonstrated that, umbilical cord and heart derived MSCs expressed identical levels of surface antigens such as CD90, CD44, CD105 and CD73. The present immunophenotyping pattern of MSCs derived from umbilical cord and human heart were found to be consistent with the reports of other groups (Hoogduijn et al. 2007; Park et al. 2007; Bai et al. 2007). In addition,

we have also demonstrated that MSCs derived from two different sources have the ability to undergo multilineage mesenchymal differentiation such as osteogenic and adipogenic differentiation (Can and Balci 2011; Hoogduijn et al. 2007).

Ion channels play a major role in cellular electrophysiological activities by regulating the potential of membranes. The electrophysiological activity also facilitate the ions movement across the membrane to create the desired intra-cellular electrical gradient (Cesare et al. 1999). In this context, it is essential to study the nature of electrophysiological properties of stem cell for regenerative medicine. Especially in cardiac regeneration, certain stem cells that exhibit electrophysiological changes might impose an arrhythmic risk by negatively regulating the natural electrical conductance (Peters 2005; Menasche et al. 2003; Siminiak et al. 2004). Thus, in this context, we have demonstrated the presence of ion channels in both MSCs at the mRNA levels. Accordingly, three types of K+ channels (IKDR, IKCa and Ito) and one type of Na+ (INa.TTX.) channel expression was detected in both groups of hUC-MSCs and hC-MSCs. The presence of K^+ channel subunits are responsible for membrane potential through delayed rectifier K^+ current (IKDR), Ca^{2+} -activated K^+ current (IKCa) and transient outward K^+ current (Ito). As well, the presence of Na+ channel is responsible for inward sodium current (Bai et al. 2007; Park et al. 2007).

Our RT-PCR results confirmed the presence of four types of ion channels in all tested MSCs, although the relative mRNA expression levels of ion channel subunits were not similar between hUC-MSCs and hC-MSCs. We found higher gene expression levels of delayed rectifier-like K+ current (Kv1.1, Kv2.1, Kv1.5) ion channel subunits in hUC-MSC than those of hC-MSC. In contrast, mRNA expression level of Ca^{2+}-activated K+ current channel subunits (KCNN3 and KCNN4) was significantly increased in hC-MSC when compared to hUC-MSC. The expression levels of other two types of channels, transient outward K+ current (Ito) and TTX-sensitive transient inward sodium current (INa.TTX) encoding genes (Kv4.2, 615 Kv4.3 and hNE-Na, respectively) were comparable between both hUC-MSCs and hC-MSCs. In line with other studies, the mRNA expression levels of inward and outward ion channels were not comparable among MSCs from different sources. The possible explanation of such slight variation in ion channel expression between hUC-MSC and hC-MSC may be the different origin or age of sample source or percentage of committed progenitor cells.

Next, we have used human heart tissue as a positive control to compare the ion channel expression levels with undifferentiated MSCs from two different sources. Our results showed that all three types of K + channel subunits of mRNA expression were almost equal or greater in human heart tissue than those of MSCs from different sources. Surprisingly, TTX-sensitive transient inward sodium current gene expression was down regulated in human heart tissue when compared to undifferentiated MSCs from two different origins. These results suggest that differentiated cardiomyocytes have stronger or better defined ion channels than undifferentiated MSCs. Surprisingly, TTX-sensitive transient inward sodium current gene expression was down regulated in human heart tissue when compared to undifferentiated MSCs from two different origins. The speculation on TTX-sensitive Na^+ channels function was clarified by comparing Na^+ channels in the heart of various mammals including human. The TTX-sensitive Na^+ channels are most likely not functionally expressed in the heart of higher mammals (Zimmer et al. 2014). In line, our data also confirmed that, the expression of TTX in heart biopsy is lower than those expressed in undifferentiated MSCs.

In summary, the present study demonstrated the similarities and differences between resident cardiac derived mesenchymal stem cells and non-resident umbilical cord derived mesenchymal stem cells, in term of proliferation, surface antigen expression and ion channel expression profiles. However, hUC-MSC have added advantage with sample availability, stability in prolonged culture, proliferation capacity in later passages. Thus, hUC-MSC could be considered as a potential alternative source for cardiac-regeneration. The characterization of ion channel expression in undifferentiated MSCs provides a fundamental understanding for further assessment of the safety issues of stem cell based therapy, not only for treatment of heart disorder, but also for other possible biological application in the future.

Limitation

The potential limitation of this study is that we used aged heart biopsy tissue to compare with newborn umbilical cord. There could be variation in their

genetic expression, mutation level or other epigenetic factors. Only gene expression profiling was performed and not the protein expression.

Compliance with ethical standards

Conflict of interest The authors express no conflicts of interest towards the publication of this paper.

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