UNIVERSITI PUTRA MALAYSIA

POTENTIAL DIFFERENTIATION OF HUMAN AMNIOTIC EPITHELIAL STEM AND MESENCHYMAL BONE MARROW CELLS INTO CARDIOMYOCYTES

BATOOL MOTAMEDI

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By

BATOOL MOTAMEDI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2015

DEDICATION

Dedicated to the great soul of my father

My Lovely Grand Mother &My Lovely Mother

My Kind Brother Mehdi

And

All My Sincere Sisters

Fatemeh

Zahra

Tayebeh

Hoda

Abstract of Thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the Degree of Doctor of Philosophy

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Chairman : Professor Dato' Tengku Azmi bin Tengku Ibrahim, PhD
Institute : Biosciences

Coronary artery disease (CAD) is the leading cause of cardiovascular mortality worldwide and stem cell transplantation is one of the approaches in the treatment of CAD (Leri *et al.*, 2008; Laflamme and Murry, 2011).Realization of regenerative cardiac medicine is dependent on the availability of cardiomyocytes in sufficient numbers for transplantation. Bone marrow mesenchymal stem cells (BM-MSCs) have been used in clinical cell therapy and injecting BM-MSCS into mouse with Kaposiôs sarcoma resulted in reduced tumour. However the same cells have been reported to promote tumour growth when injected in mouse with osteosarcoma which also promoted pulmonary metastasis.

The amnion is a simple epithelium resting on a connective tissue layer comprising of collagen fibers and fibroblasts. The amnion has two groups of stem cells. The first group are surface epithelial cells with pluripotent properties; the second group are stromal cells with mesenchymal stem cells (MSCs) properties in the underlying connective tissue of the amnion. Pluripotent cells are capable of giving rise to various body cell types similar to those of the three germ layers of the early embryo. MSCs have at least three primary characteristics: these cells grow as adherent cells in tissue culture dish; have a life span of 30 to 50 population doubling number and *in vitro* these cells could differentiate into osteoblasts, chondroblasts, and adipocytes.

In view of the above amnion which form part of the placenta and discarded following child birth is therefore a useful biological material as it is a source of cells for transplantation. In the search for a source of cardiomyocytes for transplantation the present study investigates into the characteristics and potential of human amniotic epithelial cells (hAECs) to differentiate into cardiomyocytes. The characteristics and differentiation potential of the hAECs are concurrently compared with that of BM-MSCs, the gold standard in cell therapy.

hAECs and BM-MSCs were isolated from the amniotic membrane and bone marrow, respectively and their cell surface antigens characterized based on flow cytometry, culture properties and colony formation. The proliferation rates of hAECs and BM-MSCs were calculated based on population doubling time, while adipogenic and osteogenic differentiation potentials were confirmed by the oil red O and alizarin red S staining methods, respectively. In addition, alkaline phosphatase (ALP) activity was determined using a colorimetric assay kit. The hAECs and BM-MSCs were then differentiated into cardiomyocytes in a cardiogenic medium containing 3µM 5-azacytidine. The differentiated cardiomyocyte were compared with normal cardiomyocytes by focusing on their specific protein expressions while their structural properties were determined by transmission electron microscopy.

Results showed that both hAECs and BM-MSCs expressed MSCs factors. The expression of CD73, CD105, and CD90 in hAECs was 77.3%±4.9%, 78.3%±7.2% and 87.7%±3.1% respectively while in the BM-MSCs the expression for the same factor was 82%±4%, 80%±8.3% and 83.3%±2.9%, respectively. hAECs and BM-MSCs on the other hand did not express hematopoietic stem cell factors. The expression of CD34 and CD45in hAECs was 6%±1.1% and 5.5%±1.5% respectively while in the BM-MSCs expression for the same factor was 4.5%±0.5% and 5%±1%, respectively. No significant differences in mesenchymal and hematopoietic stem cells factors expression was observed between the two groups of cells. The low expression of CD34 and CD45 in the hAECs and BM-MSCs showed that these cells were not contaminated with cord blood or embryo or bone marrow hematopoietic cells.

Results from the present study also demonstrated that the hAECs expressed embryonic stem cell markers where the expression of OCT4 in these cells was 73%±11% while in the BM-MSCs the expression for the same marker was 19%±2%; there was thus a significant difference between the two cell types *($p<0.001$)*. From these data it can be deduced that the hAECs are pluripotent while the BM-MSCs are multi potent with the potential to differentiate into derivatives of two germ layers.

Both hAECs and BM-MSCs could possibly differentiate into adipogenic cells as indicated by the positive Oil Red O staining (over 70% and 50% respectively). Both cell types also demonstrated the potential to differentiate into osteogenic cells as evidenced by positive alizarin red staining (over 35% and 55%, respectively) with significant difference (*$p<0.01$*) between the two cell types. hAECs one week after primary culture and BM-MSCs two weeks after primary culture formed colonies with alkaline phosphatase activities.

The use of 5-Azacytidine at 3µM concentration demonstrated that both hAECs and BM-MSCs could possibly differentiate into cardiomyocytes. Based on this observation on their differentiation potential the structural organization of both cell types were examined at the ultra-structural level. These differentiated cells showed initially the formation of unorganized myofibrils and subsequently to organized and parallel myotubes. The genotypes of these cells were determined by immunocytochemistry staining. The antibodies against alpha-actin, connexin43, N-cadherin, desmin, nestin and vimentin were used to identify differentiated cells. The induced hAECs and BM-MSCs expressed specific cardiomyocyte proteins in the form of alpha-actin (55% and 44% respectively) and connexin43 (80% and 70% respectively) which were similar to that of normal cardiomyocyte. The quantitative real-time RT-PCR indicated that there were no statistical significant differences between the expression of GATA-4, MLC-2a, MLC-2v, cTnI and connexin-43 in induced hBM-MSCs and hAECs with neonatal heart tissue.

From the above result it can be concluded that the hAECs possess stem cells properties similar to that of BM-MSCs and express some pluripotent and embryonic markers at a level higher than BM-MSCs. In addition hAECs are similar to BM-MSCs where, both groups that of could differentiate into osteocyte and adipocyte; however, hAECs have a greater the tendency to differentiate into adipocyte, while BM-MSCs have a greater the tendency to differentiate into osteocyte. When these cells are induced into cardiomyocyte both hAECs and BM-MSCs showed similarities to cardiomyocytes such as the formation of unorganized myofibrils to organized, parallel myotubes and expressed cardiomyocyte specific genes and protein markers. Hence, hAECs could be a suitable substitute in heart cell transplantation instead of BM-MSCs.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah.

KEUPAYAANPEMBEZAAN STEM EPITELIUM AMNION DAN SEL MESENKIM SUM SUM TULANG KEPADA KARDIOMIOSIT

Oleh

BATOOL MOTAMEDI

Disember 2015

Pengerusi : Professor Dato' Tengku Azmi bin Tengku Ibrahim, PhD
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Penyakit arteri koronari (CAD) merupakan penyebab utama mortaliti kardiovesel diseluruh dunia dan pemindahan sel stem adalah salah satu pendekatan dalam rawatan CAD (Leri *et al.*, 2008; Laflamme and Murry 2011). Kejayaan perubatan kardium regenerative bergantung kepada bilangan kardiomiosit yang mencukupi untuk pemindahan. Sehubungan dengan ini sel stem mesenkim sum sum tulang (BM-MSCs) telah digunakan untuk terapi sel klinikal dan suntikan sel tersebut kepada tikus mempunyai sarcoma Kaposisi berakhir dengan pegurangan saiz tumor. Namun demikian sel sel yang sama apabila disuntik kepada tikus mempunyai ostesarkoma telah dilaporkan mepromosi tumbesar kanser tersebut disamping mempromosi metastasis pulmonari.

Amnion adalah epitelium ringkas yang terdapat pada permukaan lapisan tisu penyambung yang terdiri daripada gentian kolagen dan fibroblast. Amnion mempunyai dua jenis sel stem. Jenis pertama adalah sel epitelium permukaan dengan ciri ciri pluripoten; jenis kedua adalah sel stroma bercirikan sel stem mesenkim (MSCs) yang terdapat dalam tisu penyambung dibawah epiteliumamnion. Sel pluripoten berupaya menghasilkan pelbagai jenis sel badan sama seperti keupayaan sel sel tiga lapisan germa di peringkat awal embrio. Sel MSC mempunyai sekurang kurangnya tiga ciri utama : dalam piring tisu kultur sel sel tersebut tumbesar sebagai sebagai sel adheren, mempunyai jangka hayat antara 30 hingga 50 lipatganda populasi sel dan *in vitro* sel sel ini boleh membeza menjadi osteoblast, kondroblast dan adopsit.

Berasaskan di atas amnion yang membentuk sebahagian daripada uri dan terbuang selepas kelahiran bayi merupakan bahan biologikal amat berguna sebagai sumber sel untuk transplantasi. Dalam usaha mencari sumber kardiomiosit untuk tujuan transplantasi kajian ini menyelidik ciri-ciri dan keupayaan sel epitelium amnion maknusia (hAEC) untuk membeza menjadi kardiomiosit. Ciri-ciri dan keupayaan membeza sel hAEC dikaji serentak bersama dengan sel BM-SC - piawai emas dalam terapi sel.

Dalam kajian ini sel sel hAEC dan BM-MSC telah diasingkan masing masing daripada membran amnion dan sum sum tulang. Antigen permukaan sel sel tersebut dicirikan berasaskan sitometri alir, sifat-sifat kultur dan pembentukan koloni. Kadar proliferasi sel sel tersebut dihitung berasaskan tempoh lipatganda populasi sementara keupayaan embezaan adipogenik dan osteogenik disahkan menggunakan kaedah pewarnaan Oil Red O dan Alizarin Red S. Di samping itu aktiviti fosfates alkalin (ALP) disahkan menggunakan kit kolometri. Sel sel hAEC dan BM-MSC seterusnya diperbezakan menjadi kardiomiosit dalam medium mengandungi 3µM 5-azacytidine. Sel sel hAEC dan BM-MSC yang diperbezakan dibandingkan dengan kardiomiosit normal dengan tumpuan diberikan kepada

ekspresi protein khusus sementara sifat struktur sel disahkan secara mikroskopi elektron transmisi.

Hasil kajian menunjukkan sel sel hAEC dan BM-MSC mengekspres faktor faktor sel MSC. Ekspresi CD73, CD105 dan CD90 dalam sel hAEC masing masing adalah 77.3%±4.9%, 78.3%±7.2% dan 87.7%±3.1% sementara dalam sel BM-MSC ekspresi bagi faltor yang sama masing masing adalah 82%±4%, 80%±8.3% dan 83.3%±2.9%. Namun demikian sel sel hAEC dan BM-MSC tidak mengekspres faktor sel stem hemapoiesis. Pengekspresan CD34 dan CD45 dalam sel hAEC adalah masing masing 6%±1.1% dan 5.5%±1.5% sementara dalam sel BM-MSC ekspresi untuk faktor yang sama adalah masing masing 4.5%±0.5% dan 5%±1%. Tiada perbezaan signifikan antara ekspresi faktor sel mesenkim dan hemapoiesis yang dapat di cerap antara dua kumpulan sel tersebut. Ekspresi CD34 dan CD45 yang rendah dalam sel sel hAEC dan BM-MSC menunjukkan bahawa sel sel ini tidak dicemari oleh sel tali pusat, sel embrio atau sel hemapoiesis sum sum tulang.

Hasil daripada kajian ini juga menunjukkan sel hAEC mengekspres penanda sel stem embrionik dan ekspesi OCT4 dalam sel sel ini adalah 73%±11% sementara dalamsel BM-MSC ekspresi bagi penanda yang sama adalah 19%±2%. Dengan demikian terdapat perbezaan signifikan antara dua kumpulan sel *(p<0.001)*. Daripada data ini dapat dirumuskan sel AEC adalah pluropoten sementara sel BM-MSC adalah multipoten dengan keupayaan membeza kepada dua daripada tiga lapisan germa.

Kedua dua sel hAEC dan BM-MSC berkemungkinan dapat membeza kepada sel adipogenik seperti yang ditunjukkan oleh pewarnaan positif Oil Red O (masing masing melebihi 70% dan 50%). Kedua jenis sel juga menunjukkan keupayaan membeza kepada sel osteogenic seperti terbukti daripada pewarnaan positif alizarin merah *(p<0.01)*. Sel hAEC selepas satu minggu kultur primer dan sel BM-MSC dua minggu selepas kultur primer kedua duanya membentuk koloni dengan aktiviti fosfatas alkalin.

Penggunaan 5-Azacytidine pada konsentrasi 3µM memberi bukti sel hAEC dan BM-MSC berkemungkinan boleh membeza menjadi kardiomiosit. Berasaskan kepada keupayaan membeza ini kedua dua jenis sel ini telah diperiksa diaras ultrastruktur. Sel sel terbeza ini pada mulanya menunjukkan pembentukan miofibril tidak tersusun dan seterusnya membentuk miotubules yang tersusun dan selari. Genotip sel sel ini telah disahkan secara pewarnaan imunositokimia. Antibody penentang alfa-aktin, konexin-43, N-kadheri, desmin, nestin dan vimentin telah digunakan untuk mengenalpasti sel sel terbeza. Sel sel hAEC dan BM-MSC teraruh mengekspres protein kardiomiosit khusus dalam bentuk alfa-aktin masing masing 55% dan 44%) dan koneksin-43 (masing masing 80% dan 70%) yang juga terdapat pada kardiomiosit normal. Kuantatif masa-sebenar RT-PCR menunjukkan tiada perbezaan signifikan antara pengeksperesan GATA-4, MLS-2a, MLC-2v, cTnI dan kontksin-43 dalam sel sel hAEC dan BM-MSC teraruh dengan tisu jantung neonat.

Kesimpulan yang dapat diambil daripada kajian ini ialah sel hAEC mempunyai sifat sifat sama seperti yang terdapat pada BM-MSC dan mengekspres penanda pluripotent dan embrio pada aras yang lebih tinggi berbanding dengan BM-MSC. Di samping itu sel hAEC adalah sama seperti sel BM-MSC dari segi kedua dua kumpulan sel boleh membeza menjadi osteosit dan adiposit; namun demikian lebih banyak sel hAEC mempunyai kecenderungan untuk membeza menjadi adiposit sementara lebih banyak sel BM-MSC membeza menjadi osteosit. Apabila sel ini teraruh menjadi kardiomiosit ianya menunjukkan persamaan dengan kardiomiosit dari segi pembentukan miofibril tidak tersusun kepada miotubules yang tersusun dan selari dan mengekspres protein khusus dan gen. Dengan demikian hAEC mungkin membentuk substitut yang sesuai dalam transplatasi sel jantung berbanding dengan sel BM-MSC.

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I certify that a Thesis Examination Committee has met on 17 December 2015 to conduct the final examination of Batool Motamedi on her thesis entitled "Potential Differentiation of Human Amniotic Epithelial Stem and Mesenchymal Bone Marrow Cells into Cardiomyocytes" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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AE	Amniotic Epithelium
AFP	Alpha Feto-Protein
Alb	Albumin
ALP	Alkaline Phosphatase
AM	Amniotic Mesoderm
ANF	Atria Natri-uretic Factor
AST	Aspartate transaminase
ATP	Adenosine Triphosphate
A-V	Atria-Ventricular
BM	Bone Marrow
BM-MSCs	Bone Marrow Mesenchymal Stem Cells
BM-SCs	Bone marrow stem cells
BMPs	Bone Morphogenetic Proteins
BNP	B-type natriuretic peptide
CAD	Coronary Artery Disease
CCR	Chemokine Receptor
CD	Cluster of Differentiation
CM	Chorionic Mesoderm
CMG	Cardiomyogenic
CPCs	Cardiac Progenitor Cells
CSCs	Cardiac Stem Cells
CSF	Cerebro Spinal Fluid
cTnI	cardiac Troponin I
CT	Chorionic Trophoblast
CX-43	Connexin-43
DAB	Di-Amino-Benzidine
DMEM	Dulbeccoôs Modified Eagleôs Medium
EDTA	Ethylene Diamine Tetraacetic Acid
EGF	Epidermal Growth Factor
EMT	Epithelial to Mesenchymal Transition
ESCs	Embryonic Stem Cells
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
FCS	Fetal Calf Serum
FITC	Fluorescein Iso-Thio-Cyanate
GAPDH	Glycer-Aldehyde 3-Phosphate De-Hydrogenase
GFAP	Glial Fibrillary Acidic Protein
GSK	Glycogen Synthase Kinase
hAECs	human Amniotic Epithelial Cells
hAMSCs	human Amniotic Mesenchymal Stromal Cells
hBM-MSCs	human Bone Marrow Mesenchymal Stem Cells
hCMSCs	human Chorionic Mesenchymal Stromal Cells
hCTCs	human Chorionic Trophoblastic Cells
hESC	human Embryonic Stem Cell
HG	High Gravity
HGF	Hepatocyte Growth Factor
HLA	Human Leukocyte Antigen
IL	Interleukin
KMU	Kerman Medical Sciences University
LDH	Lactate dehydrogenase
LIF	Leukemia Inhibitory Factor
MAPCs	Mesenchymal Adult Progenitor Cells

MG	Micro Gravity
MLC-2a	atrial Myosin Light Chain 2
MLC-2v	ventricular Myosin Light Chain 2
MSCs	Mesenchymal Stem Cells
MyoD	Myosin D
NF-H	Neuro Filament- Heavy chain
OCT-4	Octamer-Binding Protein 4
PBS	Phosphate-Buffered Saline
PE	Phyco-Erythrin
PDGF	Platelet-Derived Growth Factor
PDT	Population Doubling Time
rhEGF	recombinant human Epidermal Growth Factor
S-A	Sino-Atrial
SCI	Spinal Cord Injury
SCID	Severe Combined Immuno-Deficiency
SSEAs	Stage-Specific Embryonic Antigens
TGF	Transforming Growth Factor
TRAs	Tumor Rejection Antigens
vWF	Von Willebrand factor
W.H.O	World Health Organisation

CHAPTER 1

INTRODUCTION

Heart disease is one of the most common health problems. According to the World Health Organization (WHO), cardiovascular disease is the leading cause of death worldwide (Feigin *et al.*, 2014). Dysfunction of heart muscle cells or myocardial infarction (MI) occurs among 1.1 million Americans each year (Leri *et al.*, 2008). The estimated incidence of Acute Coronary Syndrome (ACS) is 141 per 100,000 populations per year, and the inpatient mortality rate is approximately 7% in Malaysia (National Cardiovascular Disease Database). And 50 percent of all deaths per year in Iranian population concerned to coronary artery disease (Hatmi *et al.*, 2007). The increasing prevalence of human chronic diseases such as cardiovascular disease, presents a challenge to find more effective therapies (Zhao *et al.*, 2006).

The myocardium possesses cardiomyocytes or cardiac muscle cells. Each myocardial cell contains myofibrils consisting of long chains of sarcomeres, which are the fundamental muscle cell contractile units. Cardiomyocytes exhibit striations similar to that observed in skeletal muscle cells and have only one nucleus. Cardiomyocytes contain a high mitochondrial density to produce adenosine triphosphate (ATP), making these cells highly resistant to fatigue (Sarantitis *et al.*, 2012). Any condition which reduces the efficiency of the myocardium leads to heart failure. This condition include myocardial infarction where there is oxygen deficiency in the heart muscle leading to the death of these cells; hypertension which increase the force of contraction to pump blood and makes the heart muscle inflexible (Boron and Boulpaep, 2012).

During embryogenesis, heart mass increases mainly through the division of cardiomyocyte cells, a process referred to as hyperplasia; cardiomyocytes do not regenerate after birth and they react to mitotic signals by increasing the cell size, i.e. hypertrophy rather than hyperplasia (Pan *et al.*, 1999; Niu *et al.*, 2013). Cardiac myocytes rapidly proliferate in the fetal life but in the prenatal period, proliferation ceases and myocytes could undergo additional incomplete mitosis where karyokinesis takes place in the absence of cytokines, leading to bi-nucleation in most species. Typically, adult cardiac myocytes exposed to growth signals do not reenter the cell cycle and by hypertrophy led to increase in cardiac mass. As a result, cardiac myocytes demonstrate three forms of growth; bi-nuclear cell, proliferation, and hypertrophy (Ahuja *et al.*, 2007). Cardiac hypertrophy is also induced by humoral factors and mechanical load, such as nor epinephrine, angiotensin-II (Ang-II), and endothelin-1 (ET-1). Mechanical stretch is one of the most important stimuli of cardiac hypertrophy. Mechanical stretch-induced signal transduction is characterized by simultaneous activation of multiple second messenger systems (Zablocki and Sadoshima, 2013; Ward *et al.*, 2014). Myocardial

regeneration makes repopulation of irreversibly damaged muscle with new contractile cells to restore functionality in the necrotic areas, thereby improving global heart function. Ideally, these cells come from the spared peri infarct myocardium. Until recently, it was thought that adult mammalian cardiomyocytes were terminally differentiated, which could not divide. With ischemic and dilated cardiomyopathies some cells could recycle. However, the magnitude of this self-repair mechanism is by far too limited to compensate for the massive loss of cardiomyocytes resulting from a large infarct.

Although, genetically induced conversion of in-scar fibroblasts into myogenic cells has little clinical applicability the only practicable perspective is an exogenous supply of cells for effecting re-population of injured areas. Ideally, exogenous cells should possess the following criteria: 1) easy to collect and expand; 2) form stable intra myocardial grafts; 3) have the ability to electromechanically couple with host cardiomyocytes to beat synchronously; and 4) lack of arrhythmogenic and oncogenic effects.

Using fetal and neonatal cardiomyocytes revealed that these cells were able to engraft successfully into infarcted myocardium and express gap junction proteins, survive for long periods and improve left ventricular function. However, the use of fetal tissue is fraught with major availability immunogenicity, and ethical issues (Leor *et al.*, 1996). Following myocardial infarction, functional recovery is dependent on increasing blood flow and regeneration of tissue. A study showed that transplanted fetal cardiomyocytes can survive in scar tissue as these cells possess angiogenesis properties and the transplanted cells limit scar expansion and prevent post infarction heart failure (Leor *et al.*, 1996). In this respect, cellular cardiomyoplasty has emerged as an alternative treatment in the regeneration of infracted myocardial tissue and different cell lines (e.g. cardiomyocytes, skeletal myoblasts, and adult mesenchymal stem cells), resulting in an improvement in ventricular function and decrease in the amount of infracted tissue (Aceves *et al.*, 2005).

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed a minimal set of criteria to define human mesenchymal stem cells (MSCs) for research (Dominici *et al.*, 2006): It should retain the ability to adhere to plastic when maintained under standard culture conditions; it should express CD105, CD73 and CD90 as MSCs factors, but not express the hematopoietic markers such as CD45, CD34, CD14, or CD11b, CD79a or CD19 and HLA-DR surface molecules; and finally it should be able to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro* (Roorda *et al.*, 2009).

Stem cells (SCs) could provide precursors of cells for cardiomyocyte differentiation, endothelial and supporting cells in addition to making signals for cell activation and obstacle of apoptosis. Human bone marrow mesenchymal stem cells (hBM-MSCs) are currently being utilized in clinical therapy trials of patients with heart failure of ischemic and non-ischemic etiologies (Hoover-Plow and Gong, 2012). Although hBM-MSCs are used in clinical cell therapy Khakoo *et al.* (2006) revealed in their study that injecting hBM-MSCs into mouse with Kaposi's sarcoma resulted in a reduced tumour. However, some cells have been reported to promote tumour growth when injected in mouse with osteosarcoma and also promoted pulmonary metastasis (Xu *et al.*, 2009). Controversy remains as to whether hBM-MSCs are the best cells to be used for replacement therapy. The injection of hBM-MSCs improves the performance of the pathological heart but the mechanism by which the administration of hBM-MSCs results in enhanced cardiac function remains controversial (Leri *et al.*, 2008). Although adult SCs can be directly isolated from the patient and are therefore immunologically compatible isolation culture and growing these cells are difficult. In contrast, human embryonic stem cells (hESCs) can proliferate very rapidly in culture and differentiate into cells of all adult tissues. Initial studies with hESCs-derived cardiomyocytes have shown their capacity to form new myocardium in the uninjured heart (Laflamme *et al.*, 2005; Xue *et al.*, 2005). One hopeful aspect of these cells is that they will be able to achieve at least a degree of electrical integration with adjacent host myocardium in such models (Kehat *et al.*, 2004; Léobon *et al.*, 2003). In the infracted rat heart, hESCs-derived cardiomyocytes improved its cardiac structure and function (Laflamme *et al.*, 2007; Ardehali *et al.*, 2013). However, there is a need to control the growth and overcome the risks of tumour formation and graft rejection by undifferentiated hESCs. It is also pertinent to resolve the ethical issues surrounding the use of materials from embryos. On the other hand, transplantation of a sufficient number of cells to adult tissue needs a large-scale cell supply. Therefore, attention is focused on obtaining a new source of stem cells (Toda *et al.*, 2007).

The cell layer of the amnion with stem cell characteristics was examined by Toda and colleagues (2007) focusing on mesenchymal stromal cells and their results indicated that the amnion contains significant plasticity and differentiation potential into cardiomyocytes. Amniotic and chorionic membranes are normally found at the edge of the placenta and the amnion covering the embryo. Human amniotic epithelial cells (hAECs) constitute the inner layer of the amnion and are formed from the amnioblast on the eighth day after fertilization. It has been proposed that hAECs could have the potential to differentiate into a wide variety of different cells including cardiomyocytes (Okrainec *et al.*, 2004).

The amniotic membrane that belongs to the placenta is normally discarded after parturition. Without harming mothers or babies stem cells can be easily isolated from membrane and there are no ethical issues associated with its isolation versus

taking hBM-SCs using biopsy and aspiration procedure. hAECs are useful biological material and also a novel cell source for cell transplantation (Paracchini *et al.*, 2012). Amniotic membranes have been applied in the treatment of burnt lesion, surgical wound (Trelford and Trelford-Sauder, 1979), and ocular surface reconstitution. Furthermore, amnion-derived cells have considerable advantages in terms of low immunogenicity and anti-inflammatory functions (Sivakami *et al.*, 2007); moreover the cells do not require the sacrifice of human embryos for their isolation, thus avoiding the current controversies associated with the use of human ES cells (Toda *et al.*, 2007). Under high-density culture conditions, amniotic epithelial cells form spheroid structures and retain stem cell characteristics. The cells express Oct-4, SSEA-3, TRA1-60, or TRA1-81 and have the potential to differentiate *in vitro* into all three germ layers: endoderm (liver, pancreas), mesoderm (cardiomyocytes), and ectoderm (neural cells). However, long-term self-renewal ability and single-cell clonal analysis will be necessary before describing them as stem cells (Toda *et al.,* 2007). AE cells express Oct-4 and nanog, transcription factors. Among molecular stem cell markers, Oct-4 plays a critical role in maintaining pluripotency and self-renewal. Though AECs are pluripotent, these cells do not form teratomas upon transplantation into the testes of SCID mice (Ilancheran *et al.*, 2007; Miyamoto *et al.*, 2004). It shows that AECs have low immunogenicity and would therefore have a reduced risk of rejection upon transplantation (Niknejad *et al.*, 2008).

Numerous human deliveries occur in the world daily. Placentas and associated membranes which are often discarded after child birth can be utilized to serve as good cell sources capable of yielding good cell numbers. Comparison has to be done on their characteristics and similarities to gold standard cell sources such as those from bone marrow which are currently utilized in cell therapy. Such comparisons will attract further studies in the possible application of these placental cells in clinical cell therapy. As such, this study focuses on biological and biochemical characterization (isolation and the expression of stem cell markers, colony formation and proliferation) of hAECs alongside their comparison with hBM-MSCs, as gold standard. Cell ability to differentiate into cardiomyocytes is also investigated (see Flow Chart Appendix A) since the application of replacement therapy in heart disease requires the availability of good cells in sufficient numbers.

The general objective of this research was to compare hAECs and hBM-MSCs with regard to their characteristics, expression of stem cell markers and ability to differentiate into cardiomyocytes, simultaneously, and based on this, some specific objectives have been set as follows:

1. To isolate MSCs from human bone marrow and hAECs from amnion membrane.

2. To differentiate the hBM-MSCs and the hAECs into cardiomyocytes using 5-azacytidine.
3. To determine proteins and genes expression of cardiomyocyte in the hBM-MSCs derived cardiomyocytes and the hAECs of amnion derived cardiomyocytes
4. To compare the hAECs-derived cardiomyocytes with bone marrow MSCs-derived cardiomyocytes (structural and ultra-structural characteristics).

Some hypotheses regarding this study are:

1. Stem cells can differentiate into cardiomyocytes.
2. Human amniotic epithelial cells can differentiate into cardiomyocytes.
3. The hAECs-derived cardiomyocytes are comparable with human bone marrow MSCs-derived cardiomyocytes.

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