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ORIGINAL ARTICLE

In vitro transdifferentiation of umbilical cord stem cells into cardiac myocytes: Role of growth factors

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Abstract Recently, stem cell based cell therapy has become a realistic option to replace damaged cardiomyocytes. Most studies on stem cell transplantation therapy have focused on the use of undifferentiated stem cells. There is a strong possibility that some cardiogenic differentiation of the stem cell in vitro prior to transplantation would result in higher engraftment efficiency, as well as enhanced myocardial regeneration and recovery of heart function. In this study we aimed to define the conditions for ex-vivo differentiation of cord blood stem cells to cardiomyocytes and endothelial cells. These conditions include the combination of vascular endothelial growth factor (VEGF); basic fibroblast growth factor (FGF-2) and platelet derived growth factor AB (PDGF-AB). Forty cord blood samples were included in this work. In this work, the percentage of CD34+ cells, CD31+ cells and CD34/31+ cells in mononuclear cells (MNC) suspension was counted prior to culture (day zero), and day 10 in the different growth factor cocktails used as well as the control tube, from which the fold increase of CD34+ cells, CD31+ cells and CD34/31+ cells was calculated. Detection of cardiac troponin I in the cultured cells to confirm cardiac differentiation was done at day 10 using Mouse anti-troponin I monoclonal antibody. From the present study, it was concluded that the growth factor cocktail in protocol 2 (FGF2+VEGF+PDGF-AB) gives better in vitro trans-differentiation of stem/progenitor cells in umbilical cord blood into cardiomyocytes and endothelial cells than the cytokines cocktail in protocol 1 (FGF2+VEGF) alone.

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Introduction

Coronary artery diseases, i.e. acute myocardial infarction and ischemic cardiomyopathy, are the main causes of death in most of the developed countries and are a major socioeconomic healthcare problem. Despite improved pharmacological therapy and coronary revascularization procedures by either percutaneous coronary intervention (PCI) or coronary artery bypass surgery (CABG) there is still a major need for novel therapeutic approaches [9].

It has, therefore, been a challenge in clinical research to find new treatment modalities that aim to reduce and repair the myocardial damage and improve blood supply to the myocardium in the ischemic heart. The study of regenerative medicine with vascular growth factor and stem cell therapy within the last decennium was of great interest and has been tested in clinical trials in patients with ischemic heart disease. The aim of this study was to induce the growth of new blood vessels or the replacement of damaged myocardial cells either directly by transdifferentiation of stem cells or by a paracrine effect of cytokines secreted from the stem cells [6].

In the absence of effective endogenous repair mechanisms after cardiac injury, cell-based therapies have rapidly emerged as a potential novel therapeutic approach in ischemic heart disease. A variety of stem and progenitor cells from different sources have been examined for their potential to promote cardiac repair and regeneration. At this stage, further optimization of cell-based therapy is urgently needed, and finally, large-scale clinical trials are required to eventually proove its clinical efficacy with respect to outcomes, i.e. morbidity and mortality [15].

Recently, cord blood (CB) is considered an important source of many types of stem cells, including haematopoietic stem cells (HSCs), endothelial progenitors, mesenchymal stem cells (MSCs), very small embryonic/epiblast-like (VSEL) stem cells, and unrestricted somatic stem cells (USSC), potentially suitable for use in regenerative medicine [13].

The transplantation studies carried out have demonstrated that: (i) CB is an alternative source to the marrow of HSCs of relatively easy procurement, with no-risk for mothers or donors, and with a low risk of transmitting infectious diseases; (ii) the number of CB cells available for transplantation is the most important factor for engraftment; and (iii) some degree of HLA mismatch is tolerated [4].

As cell therapy is a promising treatment modality to improve the management of cardiovascular diseases, we performed a study to define the optimal conditions for the in vitro trans-differentiation of umbilical cord blood derived stem cells into cardiomyocytes and endothelial cells.

In the present work, the percentage of CD34+ cells and CD34+/31+ cells in MNC suspension was estimated prior to culture (day zero), and after day 10 in the cardiogenic induction media with different cocktails of cardiogenic inducers as well as the control tube, from which the fold increase of CD34+ cells and CD34+/31+ cells was calculated.

Cardiogenic differentiation was proved by the detection of cardiac troponin I in the cultured cells at day 10 by immunohistochemical staining using Mouse anti-troponin I monoclonal antibody [7].

Materials and methods

Umbilical cord blood collection

The present study included 40 umbilical cord blood samples collected from the obstetric ward of the Kasr Al-Ainy Hospital during the period from January to September 2007. Only full term, completely normal, singleton pregnancies were included in this study.

Umbilical cord blood samples were obtained from the umbilical vein with the placenta in utero after fetal delivery. The cord was cleansed with betadine (bovidine iodine) and alcohol. About 10–15 ml of cord blood was collected under completely aseptic conditions on preservative free heparin (GIBCO) in a concentration of 10 IU/ml.

Mononuclear cell isolation and culture

The umbilical cord blood was diluted 1:2 with RPMI 1640 medium (GIBCO), layed over Ficoll-Hypaque (Biochrom KG) for density gradient separation (density 1.077), and centrifuged at 1500 rpm for 30 min. Light density mononuclear cell layer (Buffy coat) was collected by a sterile Pasteur pipette, washed with Hanks solution (GIBCO) twice, and then re-suspended in RPMI 1640.

Mononuclear cell counting was performed by automated cell counters. Viability testing was determined by trypan blue and found to be more than 95% for all cases prior to culture. Ex-vivo culture was started immediately after MNC preparation.

Mononuclear cells in a concentration of 1000.000/ml were suspended in nutrient medium RPMI 1640 (800 ul, GIBCO), supplemented with FCS (20 ul/ml, Lab technology/Promega), L-Glutamine (40 ul/ml, GIBCO), antibiotic–antimycotic (20 ul/ml, GIBCO). The tissue culture tubes were incubated in 5% CO_2 at 37 °C, for 7 days then the media were changed and re-incubated to complete10 days of culture.

Forty cases were subjected to short term culture (for 7–10 days) in static liquid culture medium under the following conditions:

- Group I: Cultures with the nutrient medium devoid of cardiogenic inducers (as a control group).
- Group II: Cultures with the nutrient medium supplemented with two cardiogenic and angiogenic inducers; basic fibroblast growth factor (bFGF) (10 ng/ml, R&D Systems) and Vascular endothelial growth factor (VEGF) (20 ng/ml, R&D Systems).
- Group III: Cultures with the nutrient medium with three cardiogenic and angiogenic inducers which are bFGF (10 ng/ml), VEGF (20 ng/ml) and Platelet derived growth factor-AB (PDGF-AB) (10 ng/ml, R&D Systems) which is added on 3rd day [2].

The degree of differentiation was evaluated by the percentage of CD34+ cells and CD34+/CD31+ cells by flowcytometer.

Cell viability was determined with trypan blue exclusion test and found to be more than 90% for all culture tubes.

Detection of cardiac troponin I in the cultured cells to confirm cardiac differentiation.

Flowcytometric analysis of cord blood stem cells

Cord blood mononuclear cells before and after expansion culture were incubated with FITC-conjugated anti-CD34 antibodies (DAKO) and PE-conjugated anti- CD31 antibodies (DAKO) for 30 min at 4 °C. After two washes with phosphate buffered saline (PBS) (Sigma Aldrich) cells were subsequently analyzed on FACS scan flowcytometer (Becton Dickinson).

The results were expressed as a percentage of positive events in relation to all events acquired by the gating [11].

Immunohistochemistry for the detection of cardiac troponin I in the cultured cells

MNCs for immunostaining were prepared on cytospin slides and stained for cardiac troponin T (Mouse anti-troponin I monoclonal antibody, Biomeda) visualized with a Peroxidase labeled polymer conjugated to goat anti-mouse immunoglobulins (Dako EnVision+ System-HRP, DAB). All studies were performed in triplicate using samples from different culture preparations and the results are expressed as a percentage of peroxidase positive cells/100 cells. Control stainings were performed without primary antibody.

Statistics

Data were presented as mean ± SD. Differences were tested for statistical significance by the Student's unpaired t test. A value of $p < 0.05$ was considered significant.

Results

In the current study, we aimed to develop an appropriate differentiation medium for in vitro trans-differentiation of umbilical cord blood derived stem cells into cardiomyocytes and endothelial cells. These media included the combination of different cardiogenic inducers such as VEGF; FGF-2 and PDGF-AB.

The present study was conducted on 40 umbilical cord blood (UCB) samples collected from the Obstetric ward of the Kasr Al-Aini Teaching Hospital, Faculty of Medicine, Cairo University. Only full term, completely normal, singleton pregnancies were included. Each cord blood sample was obtained from the umbilical vein of the unborn placenta after fetal delivery.

Flow cytometric enumeration of CD34+ cells in UCB-mononuclear cell suspension at day zero and at day 10 (Table 1, Figs. 1 and 2):

Statistical comparison between the percentage of CD34+ cells prior to culture (day zero) and after 10 days in cardiogenic differentiation media is shown in Table 2.

There was a statistically significant increase in the mean value of the percentage of CD34+ cells on comparing day zero to day 10 in all the cocktails used as well as the control tube.

Table 3 shows a statistical comparison between the percentage of CD34+ cells in the control tube versus protocols 1 and 2 after 10 days in cardiogenic differentiation media.

Table 1 The percentage of CD34+ cells in mononuclear cell suspension at day zero and day 10 of ex-vivo expansion of cord blood stem cells.

Culture number	% CD34+ cells			
	Day 0	Day 10		
	Initial count	Control	Tube 1	Tube 2
Mean	4.14	6.19	14.45	9.66
SD	3.81	6.27	13.09	7.36

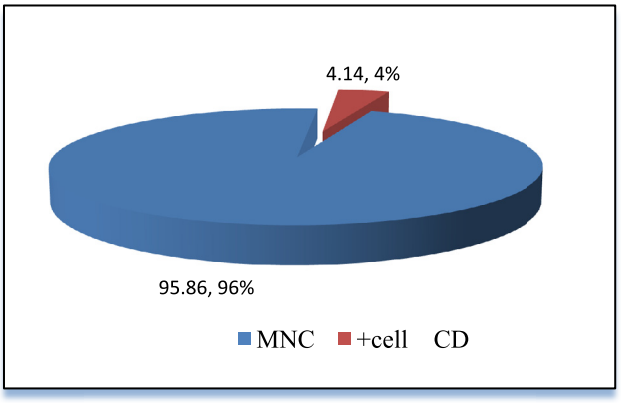

Figure 1 The percentage of CD34+ cells in MNC suspension prior to culture.

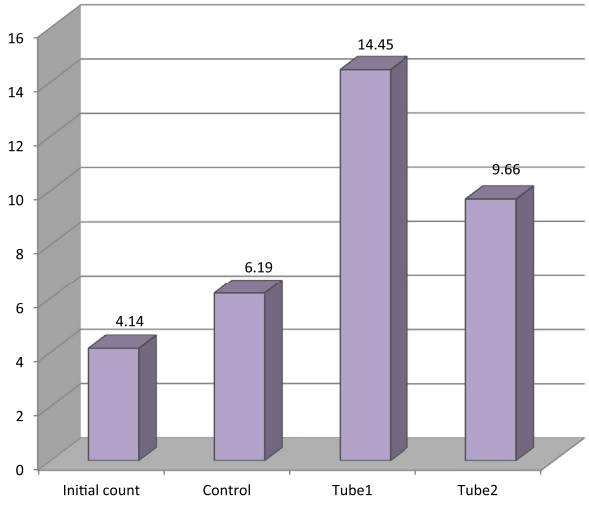

Figure 2 The percentage of CD34+ cells at day10.

There was a statistically significant increase in the mean value of CD34+ cells on comparing the control tube at day 10 to the cocktails used in tubes 1 and 2, and there was a statistically significant decrease in the mean value of CD34+ cells between protocol 1 and protocol 2.

Flow cytometric enumeration of CD34+/CD31+ cells in UCB-mononuclear cell suspension at day zero and day 10 (Table 4, Figs. 3 and 4):

Table 2 Statistical comparison between the percentage of CD34+ cells in prior to culture (day zero) and at day 10.

		Mean	Std. deviation	*p* Value
Control tube	%CD34+ cells, day zero	4.14	3.81	<0.001
	%CD34+ cells, control, day 10	6.19	6.27	
Protocol 1 (tube 1)	%CD34+ cells, day zero	4.14	3.81	<0.001
	%CD34+ cells, tube 1, day 10	14.45	13.09	
Protocol 2 (tube 2)	%CD34+ cells, day zero	4.14	3.81	<0.001
	%CD34+ cells, tube 2, day 10	9.66	7.36	

Table 3

		Mean	Std. deviation	Significance (*p* value)
Control tube versus protocol 1	%CD34+ cells, control, day 10	6.19	6.27	<0.001
	%CD34+ cells, tube 1, day 10	14.45	13.09	
Control tube versus protocol 2	%CD34+ cells, control, day 10	6.19	6.27	<0.001
	%CD34+ cells, tube 2, day 10	9.66	7.36	
Protocol 1 versus protocol 2	%CD34+ cells, tube 1, day 10	14.45	13.09	<0.001
	%CD34+ cells, tube 2, day 10	9.66	7.36	

Table 4 The percentage of CD34+/CD31+ cells in mononuclear cell suspension at day zero and day 10.

Culture number	% CD34+/31+ cells			
	Day 0	Day 10		
	Initial count	Control	Tube 1	Tube 2
Mean	3.19	4.12	8.92	6.47
SD	3.18	3.82	6.16	4.94

Statistical comparison between the percentage of CD34+/CD31+ cells prior to culture (day zero) and at day 10 (Table 5):

There was a statistically significant increase in the mean value of the CD34+/31+ cells on comparing day zero to the control tube at day 10. Furthermore, a highly statistically significant increase in the mean value of CD34+/31+ cells was noted on comparing day zero to day 10 in the control tube and the cocktails used in tubes 1 and 2.

Table 6 Comparing the mean value of the % of CD34/31+ cells in day 10 in the control tube to those of the different growth factors used showed that a highly statistically signifi-

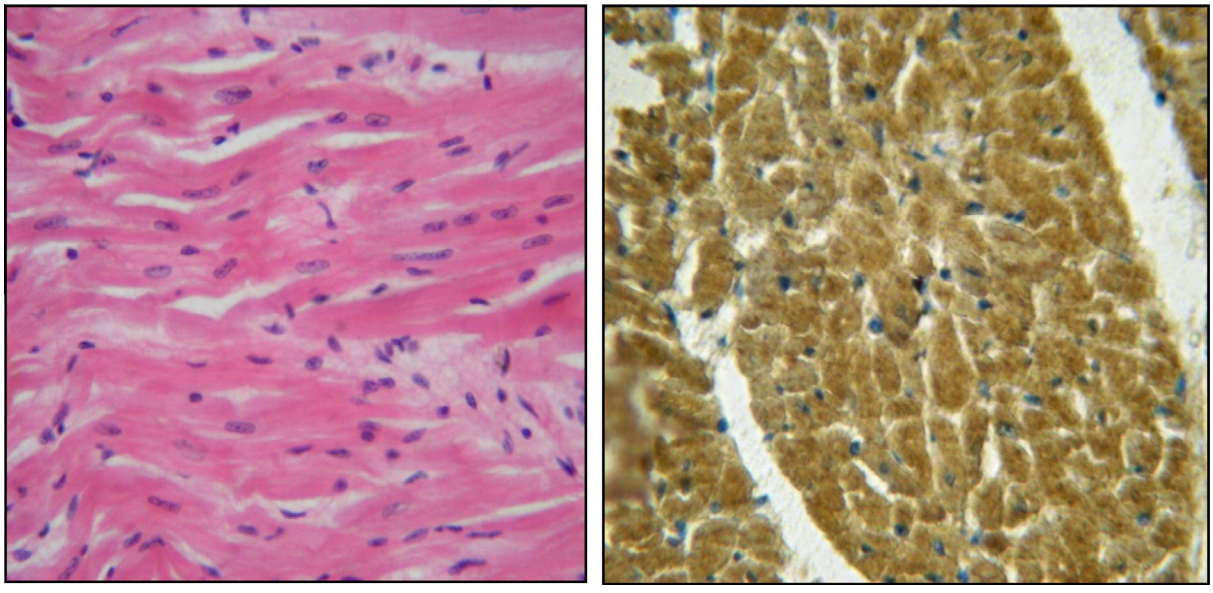

Figure 3 Human cardiac control sections stained with: (a) Haematoxilin and Eosin, (b) Mouse anti-troponin I monoclonal antibody showing Cardiac troponin I positivity (cTnI+; Brown cytoplasmic staining).

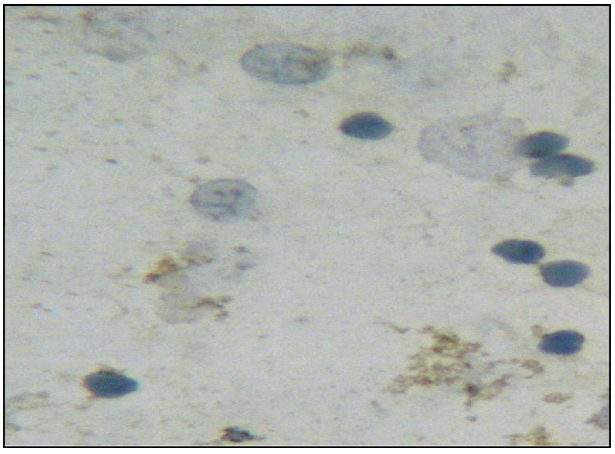

Figure 4 Immunohistochemical staining of UCB-mononuclear cells of the control tube following 10 days in culture media devoid of cardiogenic inducers. Less than 2% of the cells could differentiate into cardiomyocytes (cTnI negative cells).

cant increase in the mean value of CD34+/31+ cells was noted on comparing the control tube at day 10 to the cocktails used in tubes 1 and 2 and there was a statistically significant decrease in the mean value of CD34/31+ cells between protocol 1 and protocol 2.

The percentage of Cardiac Troponin I (cTnI) positive cells in UCB-mononuclear cell after day 10 in cardiogenic differentiation media (Table 7, Figs. 3–6.

Statistical comparison between the percentage of cTnI+ cells in the control tube versus protocols 1 and 2 after 10 days in cardiogenic differentiation media (Table 8):

Comparing the mean value of the percentage of cTnI+ cells in day 10 in the control tube to those in the different growth factors used showed a highly statistically significant increase in the mean value of cTnI+ cells noted on comparing the control tube at day 10 to the cocktails used in tubes 1 and 2 as well as between the tube 1 and the tube 2.

Discussion

Coronary artery diseases, i.e. acute myocardial infarction and ischemic cardiomyopathy, are the main causes of death in most of the developed countries and are a major socioeconomic healthcare problem. Despite improved pharmacological therapy and coronary revascularization procedures by either percutaneous coronary intervention PCI or coronary artery bypass surgery CABG there is still a major need for novel therapeutic approaches [9].

It has, therefore, been a challenge in clinical research to find new treatment modalities that aim to reduce and repair the myocardial damage and improve blood supply to the myocardium in the ischemic heart. Regenerative medicine with vascular growth factor and stem cell therapy has within the last decennium been of great interest and has been tested in clinical trials in patients with ischemic heart disease. The aim is to induce the growth of new blood vessels or the replacement of damaged myocardial cells either directly by transdifferentiation of stem cells or by a paracrine effect of cytokines secreted from the stem cells [6].

In the absence of effective endogenous repair mechanisms after cardiac injury, cell-based therapies have rapidly emerged as a potential novel therapeutic approach in ischemic heart disease. A variety of stem and progenitor cells from different sources have been examined for their potential to promote cardiac repair and regeneration. At this stage, further optimization of cell-based therapy is urgently needed, and finally, large-scale clinical trials are required to eventually proove its clinical efficacy with respect to outcomes, i.e. morbidity and mortality [15].

Table 5 Statistical comparison between the percentage of CD34+/CD31+ cells prior to culture (day zero) and at day 10.

		Mean	Std. deviation	Significance (p value)
Control tube	%CD34/31+ cells, day zero	3.19	3.18	<0.05
	%CD34/31+ cells, control, day 10	4.12	3.82	
Protocol 1 (tube 1)	%CD34/31+ cells, day zero	3.19	3.18	<0.001
	%CD34/31+ cells, tube 1, day 10	8.92	6.16	
Protocol 2 (tube 2)	%CD34/31+ cells, day zero	3.19	3.18	<0.001
	%CD34/31+ cells, tube 2, day 10	6.47	4.94	

Table 6 Statistical comparison between the percentage of CD34+/CD31+ cells in the control tube versus protocol 1 and 2 after 10 days in cardiogenic differentiation media.

		Mean	Std. deviation	Significance (p value)
Control tube versus protocol 1	%CD34+/31+ cells, control, day 10	4.12	3.82	<0.001
	%CD34+/31+ cells, tube 1, day 10	8.92	6.16	
Control tube versus protocol 2	%CD34+/31+ cells, control, day 10	4.12	3.82	<0.001
	%CD34+/31+ cells, tube 2, day 10	6.47	4.94	
Protocol 1 versus protocol 2	%CD34+/31+ cells, tube 1, day 10	8.92	6.16	<0.001
	%CD34+/31+ cells, tube 2, day 10	6.47	4.94	

Table 7 The percentage of Cardiac Troponin I (cTnI) positive cells in UCB-mononuclear cell after day 10 in cardiogenic differentiation media.

	Protocol 1 (tube 1)	Protocol 2 (tube 2)
Mean	40.05	59.18
SD	8.99	10.99

NB: the control tube contains $\leqslant 2\%$ cTnI+ cells (1.2 ± 0.8).

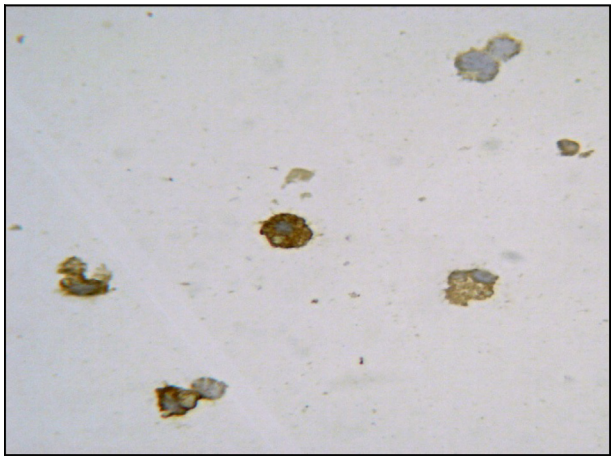

Figure 5 Immunohistochemical staining of in vitro differentiated cardiomyocytes (cTnI positive cells; Brown staining of the cytoplasm) from UCB-mononuclear cells after 10 days in cardiogenic differentiation media (protocol 1).

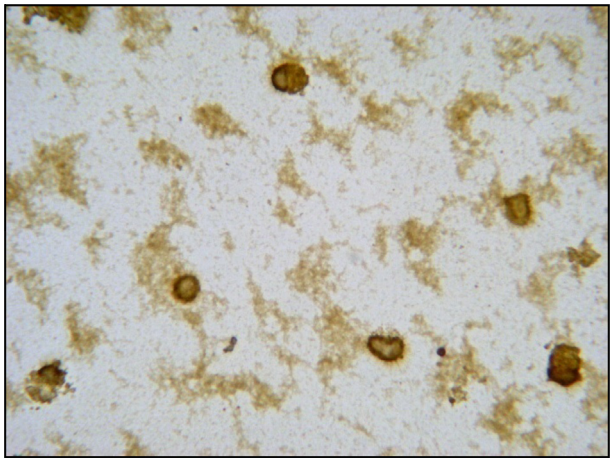

Figure 6 Immunohistochemical staining of in vitro differentiated cardiomyocytes (cTnI positive cells; Brown staining of the cytoplasm) from UCB-mononuclear cells after 10 days in cardiogenic differentiation media (protocol 2).

Recently, cord blood (CB) is considered an important source of many types of stem cells, including haematopoietic stem cells (HSCs), endothelial progenitors, mesenchymal stem cells (MSCs), very small embryonic/epiblast-like (VSEL) stem cells, and unrestricted somatic stem cells (USSC), potentially suitable for use in regenerative medicine [13].

The transplantation studies carried out have demonstrated that: (i) CB is an alternative source to the marrow of HSCs of relatively easy procurement, with non-risk for mothers or donors, and with a low risk of transmitting infectious diseases; (ii) the number of CB cells available for transplantation is the most important factor for engraftment; and (iii) some degree of HLA mismatch is tolerated [4].

As cell therapy is a promising treatment modality to improve the management of cardiovascular diseases, we performed a study to define the optimal conditions for the in vitro trans-differentiation of umbilical cord blood derived stem cells into cardiomyocytes and endothelial cells. These conditions include the combination of different cardiogenic inducers as VEGF; FGF-2 and PDGF-AB.

In this study two protocols were applied for in vitro trans-differentiation of cord blood stem cells. In protocol I, 40 cord blood samples were expanded in a static liquid culture for 10 days to which serum was added, using VEGF and FGF-2 cocktail. In protocol II, PDGF-AB was added to protocol I cocktail. This was intended to verify the effect of different cardiogenic inducers such as VEGF; FGF-2 and PDGF-AB on the trans-differentiation of umbilical cord blood derived stem cells into cardiomyocytes and endothelial cells.

In the present work, the percentage of CD34+ cells, CD31+ cells and CD34+/31+ cells in MNC suspension was estimated prior to culture (day zero), and after day 10 in the cardiogenic induction media with different cocktails of cardiogenic inducers as well as the control tube, from which the fold increase of CD34+ cells, CD31+ cells and CD34+/31+ cells was calculated.

Cardiogenic differentiation was proved by the detection of cardiac troponin I in the cultured cells at day 10 using Mouse anti-troponin I monoclonal antibody.

Basic fibroblast growth factor (bFGF or FGF-2) is a heparin-binding mitogenic protein that mediates the formation of new blood vessels and enhances the proliferation of a wide variety of cell types under serum-free or serum-reduced conditions. bFGF is also known as a repressor of myofibroblast differentiation, which would be consistent with its effects on the stimulation of Nkx2.5 expression and the consequent repression of a-SMA gene expression [10].

Hu et al. [5] showed that treatment of cells with bFGF significantly induced Nkx2.5 protein expression in a dose-dependent manner. Nkx2.5 is a transcription factor with a well known role as a promoter of cardiac cell differentiation and a suppressor of a-SMA gene expression, and thus myofibroblast differentiation.

Several studies have also demonstrated that the application of multiple growth factors for therapeutic applications e.g. VEGF, bFGF, angiopoietin-1 and PDGF has a greater impact than single growth factor delivery [14].

Vascular endothelial growth factor (VEGF) is a mitogenic and chemotactic factor for endothelial cells, and seems to play a vital role in the protection of these cells against apoptosis. The use of human VEGF as a potential stimulant in therapeutic angiogenesis has been widely demonstrated. However, there is still uncertainty as to whether the presence of human VEGF alone would suffice in the achievement of functional and mature vessels lined with vascular smooth muscles or pericytes [8].

Our study revealed that the different protocols conducted for cardiogenic differentiation showed that there was a statistically significant increase in the mean value of CD34+ cells

Table 8 Statistical comparison between the percentage of cTnI+ cells in the control tube versus protocol 1 and 2 after 10 days in cardiogenic differentiation media.

		Mean	Std. deviation	Significance (p value)
Control tube versus protocol 1	%cTnI+ cells, control, day 10	1.2	0.8	<0.001
	%cTnI+ cells, tube 1, day 10	40.05	8.99	
Control tube versus protocol 2	%cTnI+ cells, control, day 10	1.2	0.8	<0.001
	%cTnI+ cells, tube 2, day 10	59.18	10.99	
Protocol 1 versus protocol 2	%cTnI+ cells, tube 1, day 10	40.05	8.99	<0.001
	%cTnI+ cells, tube 2, day 10	59.18	10.99	

on comparing the control tube at day 10 to the cocktails using VEGF and bFGF in tube 1 and PDGF-AB in tube 2, and there was a statistically significant decrease in the mean value of CD34+ cells between the two protocols (tube 1 and tube 2).

Moreover, our study revealed that the different protocols conducted for cardiogenic differentiation showed that there was a statistically significant increase in the mean value of CD34/CD31+ cells on comparing the control tube at day 10 to the cocktails using VEGF and bFGF in tube 1 and PDGF-AB in tube 2, and there was a statistically significant decrease in the mean value of CD34/31+ cells between the two protocols (tubes 1 and tube 2).

The peptide growth factor platelet-derived growth factor (PDGF) consists of different combinations of two polypeptide chains, A and B, which form homodimeric (PDGF-AA and PDGF-BB) as well as heterodimeric (PDGF-AB) isoforms binding to two structurally related protein tyrosine kinase receptors, namely a- and b-receptors. Recently, two new PDGF isoforms, namely PDGF-CC1 and PDGF-DD2 were identified [1].

Binding to their cognate receptors results in the activation of signaling cascades that initiates proliferation, migration, and differentiation of a variety of cell types including fibroblasts and smooth muscle cells [1].

During angiogenic processes, members of the PDGF family act in concert with other pro-angiogenic factors, e.g. FGF-2, to induce angiogenic synergy and vessel stability, suggesting that combinatorial therapy with angiogenic and arteriogenic factors may be utilized in therapeutic angiogenesis [12].

Recently, it has been shown that the stimulation of PDGFR-b induced sprouting vasculogenesis in differentiating embryonic stem (ES) cells, which form capillary-like structures within the three-dimensional tissue of embryoid bodies and express the endothelial cell markers Flk-1 (VEGF-R2), flt-1 (VEGF-R1), CD31, CD34, Tie-1, Tie-2, and VE-cadherin [3].

Our findings can be summarized as follows: cytokines cocktail in protocol 2 (FGF2+VEGF+PDGF-AB) gives better in vitro trans-differentiation of stem/progenitor cells in umbilical cord blood into cardiomyocytes and endothelial cells than cytokines cocktail in protocol 1 (FGF2+VEGF) alone.

In conclusion, the use of cord blood CD34+ cells for cell-based therapy should greatly simplify the procurement of cells for the regeneration of damaged myocardium. Their use obviates the painful procedure of bone marrow aspiration and the attendant anesthesia risks. In addition, autologous UCB stem cell transplantation does not require long-term immune suppressive therapy. Thus, the use of autologous UCB stem cells for myocardial regeneration is a promising alternative for the treatment of heart failure after MI.

Summary and conclusion

The present study was intended to: (1) assess the potential use of umbilical cord blood (UCB) for reproducible differentiation into human cardiomyocytes and endothelial cells, (2) define the role of using different growth factors in vitro to enhance the differentiation capacity of UCB stem cells into cardiomyocytes and endothelial cells.

The present study included UCB samples collected from the obstetric ward of the Kasr Al-Aeini Hospital. Only full term, completely normal, singleton pregnancies were included in this study. 10–15 ml cord blood was obtained from the umbilical vein with the placenta in utero after fetal delivery.

The data presented prove the feasibility of using UCB as a source for human cardiac cells. In vitro culture of UCB with the FGF-2 and VEGF promotes the differentiation of CD34+ stem cells into endothelial cells and cardiomyocytes. Further the addition of PDGF-AB will commit the endothelial progenitor cellsCD31/CD34 transdifferentiation into cardiomyocytes.

In conclusion, the existence of cardiac specific precursors in the human umbilical cord paves the way to a possible cell therapy approach for the restoration of cardiac function after infarction, and improved knowledge on the biology of human cardiac cell differentiation.

Recommendations

Clearly there is an unmet need for medical therapies in reducing morbidity and mortality in cardiovascular diseases. The utilization of stem cells to augment or even to prevent advancing cardiovascular diseases is a subject that has great promise as a modality to address these needs. UCB is a viable source of stem cells due to its high content of CD34 and CD133 cells, as well as its robust proliferative capacity, low immunogenicity, low infectious contamination including virions, and the diverse representation of HLA genotypes present in unrelated banked UCB.

Further studies are required to develop well-defined and efficient in vitro protocols for the cardiomyogenic differentiation. This will provide the stringent levels of safety and quality control that will make the clinical applications of stem cell transplantation therapy realizable.

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