

STEM/PROGENITOR CELLS IN HUMAN UMBILICAL CORD BLOOD: PROLIFERATIVE POTENTIAL FOR IN VITRO CARDIOGENESIS AND ANGIOGENESIS

THESIS

**Submitted for Partial Fulfillment for partial fulfillment of MD Degree in Clinical
Pathology**

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2012**

ACKNOWLEDGEMENT

First of all, thanks to God for his grace and mercy, and for giving me the effort to complete this work.

I was fortunate enough to carry out this work under the supervision of Prof. Dr. Mohamed Sherif Moukhtar Professor of Cardiology and Professor of Critical Care Medicine, Cairo University. Thanks for his generous help and advice. It was a great honor to work with a great professor like him.

I would like to express my sincere thanks to Prof. Dr. Safaa Mostafa El Karaksy Professor of Clinical and Chemical Pathology, Cairo University, for her kindness, great patience and continuous support throughout the work.

Words will not be able to express my deepest gratitude and appreciation to Prof. Dr. Mervat Saad El Ansary, Professor of Clinical and Chemical Pathology, Cairo University, for her unlimited help, support and continuous encouragement.

I would like to express my deepest gratitude and thanks to Prof. Dr. Sanaa Sayed Abdel Shafy, Professor of Clinical and Chemical Pathology, Cairo University for her support, sincere help and guidance through this work.

My sincere appreciation and special thanks are due to Prof. Dr. Mervat Mamdooh Ahmed Khorshied, Assistant Professor of Clinical Pathology, Faculty of Medicine, Cairo University, for her kind supervision, continuous encouragement and generous cooperation.

I especially want to thank Prof. Dr. Heba Gouda, Assistant Professor of Clinical Pathology, Faculty of Medicine, Cairo University, Prof. Dr. Rania Hassan Khalifa, Assistant Professor of Clinical Pathology, Faculty of Medicine, Cairo University, and Prof. Dr. Inas Abdel Latif for their valuable assistance in the preparation and completion of this study.

Special thanks to all my colleagues in the Critical Care Center and the department of Clinical and Chemical Pathology for their cooperation throughout this work.

At last but not least I would like to thank the members who were behind me in every successful step in my life, and who afford me the best circumstances to realize my goal... to my family specially my great mother.

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 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 thesis 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 VEGF; FGF-2 and PDGF-AB growth factors. Forty cord blood samples were included in this work. In this work, the percentage of CD 34+ cells and CD 34/31+ cells in MNC suspension was counted prior to culture (day zero), and day 10 in the different growth factors cocktails used as well as the control tube, then from which the fold increase of CD 34+ cells and CD 34/31+ cells was calculated. Detection of cardiac troponin I in the cultured cells to confirm the cardiac differentiation was done at day 10 using Mouse anti-troponin I monoclonal antibody. From the present study, it can be concluded that 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.

Key words: cord blood- stem cells- in vitro cardiogenesis-
cardiomyocytes

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List of Abbreviations

| | |
|-------------|----------------------------------|
| Ab..... | Amyloid beta peptide |
| AD..... | Alzheimer's disease |
| ALS..... | Amyotrophic lateral sclerosis |
| AMI..... | Acute myocardial infarction |
| APC..... | Antigen presenting cells |
| ASC..... | ..Adult stem cells |
| BBB..... | Blood-brain barrier |
| bFGF..... | ..Basic Fibroblast growth factor |
| BM..... | ...Bone marrow |
| BMSCs..... | Bone marrow stem cells |
| CABG..... | Coronary artery bypass surgery |
| CB..... | ..Cord blood |
| CB-SCs..... | Cord blood stem cells |
| CI..... | ..Cerebral ischemia |
| CKD..... | Chronic kidney disease |
| CM..... | Conditioned medium |
| CMP..... | Common myeloid progenitors |
| CPCs..... |Cardiac progenitor cells |
| CSCs..... | Cardiac stem cells |
| EB..... |Epidermolysis bullosa |
| ECs..... | Endothelial cells |

EGC.....Embryonic germ cells
 EGF.....Epidermal growth factor
 EMT.....Epithelialto-Mesenchymal transition
 EO-EPCs.....Early-outgrowth endothelial progenitor cells
 EPCs.....Endothelial progenitors
 ESCs.....Embryonic stem cells
 ESRD.....Endstage renal disease
 FGF.....Fibroblast growth factor
 G-CSF.....Granulocyte colony stimulating factor
 GFP.....Green fluorescent protein
 GM-CSF.....Granulocyte/macrophage colony-stimulating factor
 GvHD.....Gravt versus host disease
 GvL.....Gravt versus leukaemia
 hASCs..... Human adipose tissue-derived stem cells
 HF.....Heart failure
 HGF.....Hepatocyte growth factor
 HMGB.....High-mobility group box protein
 HPCs.....Haematopoietic progenitors
 HSCs.....Haematopoietic stem cells
 HUVEC.....Human umbilical vein endothelial cells
 i.v.....Intravenously
 ICM.....Inner cell mass
 IGF-1.....Insulin-like growth factor-1

IL.....Interleukin
 iPS.....Induced pluripotent stem cells
 KDR..... Kinase insert domain receptor
 LIF..... Leukemia Inhibitory Factor
 LO-EPCs..... Late-outgrowth endothelial progenitor cells
 LSCD..... Limbal SC deficiency
 LV.....Left ventricular
 maGSCs..... Multipotent Adult Germline Stem Cells
 MCAO..... Middle carotid artery occlusion
 MHC..... Major histocompatibility complex
 MI.....Myocardial infarction
 MLP.....Multipotential lymphoid progenitors
 MSCs..... Mesenchymal stem cells
 NK.....Natural killer cells
 NSCs.....Neural stem/progenitor cells
 OA.....Osteoarthritis
 PCI.....Percutaneous coronary intervention
 PCNA..... Proliferating cell nuclear antigen
 PD..... Parkinson's disease
 PDGF.....Platelet derived growth factor
 PDMC..... ..Placental-derived multipotent cells
 PECAM-1.....Platelet/endothelial cell adhesion molecule-1
 PSCs.....Pluripotent stem cells
 SC.....Stem Cells

SCF..... Stem cell factor

SDF..... Stromal cell– derived factor

SJS.....Stevenson-Johnson syndrome

SM α A+..... Smooth muscle α A+

T1D.....Type1Diabetes

TNF.....Tumor necrosis factor

T β 4.....Thymosin β 4

UCB.....Umbilical cord blood

USSC..... Unrestricted somatic stem cells

VE.....Vascular-endothelium

VEGF.....Vascular endothelial growth factor

VEGFR.....Vascular endothelial growth factor receptor

VSEL..... Very small embryonic/epiblast-like

VSMCs.....Vascular smooth muscle cells.

WJ-MSCs.....Wharton's jelly Mesenchymal stem cells

Introduction and aim of the work

The ability of stem cells to renew their own population and to differentiate into specialized cell types has always attracted researchers looking to exploit this potential for cellular replacement therapies, pharmaceutical testing and studying developmental pathways (**Walia et al., 2012**).

Despite of vast improvements in treatment, myocardial infarction often leads to heart failure (HF) which remains the leading cause of death in developed countries. Other than heart transplantation, therapeutic options have a limited role in improving outcomes in patients with severe HF. It is therefore no surprise that cardiac cell therapy has raised many hopes as a novel therapeutic approach aimed at cardiac myocytes replacement - regeneration termed “cellular cardiomyoplasty”(**Pendyala et al.,2008**).

Regenerative medicine with vascular growth factor and stem cell therapy have within the last decennium had great interest and have been tested in clinical trials in patients with ischemic heart disease. The aim is to induce growth of new blood vessels or replacement of damaged myocardial cells either directly by trans-differentiation of stem cells or by a paracrine effect of cytokines secreted from the stem cells (**Kastrup, 2011**).

SCs show high plasticity, i.e. the complex ability to cross lineage barriers and adopt the expression profile and functional

phenotypes of the cells that are typical of other tissues. The plasticity can be explained by trans-differentiation (direct or indirect) and fusion (**Lodi et al., 2011**).

Stem cells have been categorized as (i) embryonic stem cells (ESCs), (ii) cord blood stem cells (CB-SCs), and (iii) adult stem cells (ASC). Each one of these populations has been characterized and further divided into sub-populations using genotyping assays and phenotypic expression of markers (**Francesse and Fiorina, 2010**).

It has been shown that CB stem cells have the ability to regenerate numerous tissue types, and when transplanted into animals and humans, have produced measurable functional improvements. Generally, tissue-derived stem cells have been described for neural, muscle, retinal, pancreas, skin and liver tissues but these tissue-specific stem cells have limited self-renewing capabilities and are unable to reconstitute a whole organ system (**Harris, 2009**).

Recently, cord blood (CB) is considered an important source of many types of stem cells, including haematopoietic stem cells (HSCs), endothelial progenitors (EPCs), 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 (**Pelosi et al., 2012**).