Therapeutic Potentials of Cord Blood Mononuclear Cells Transplantation for Limb Ischemia: a Comparison Between CD34+ and CD34- Cells.

CYTOTHERAPY
The Journal of Cell Therapy

May 2017 Volume 19, Issue 5, Supplement, Page S75

Therapeutic potentials of cord blood mononuclear cells transplantation for limb ischemia. A comparison between CD34+ and CD34– cells

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DOI: http://dx.doi.org/10.1016/j.jcyt.2017.02.115

ated cell (TNC), CD34, and also time to product. Indonesia as an archipelago country with 17,508 islands have stem cell processing lab where located only in capital city. It is very challenging to transport and finished the UCB sample processing before 48 hours.
Method & Results: We have analyzed 206 UCB units from different sites of Indonesia which had collected, processed and cryopreserved. All samples positive for Anti-HIV, HBsAg, Anti-HCV, VDRL/TPHA, CMV IgM and bacterial or fungal contamination were excluded for banking. Average processed UCB volume, TNC, and Total Viable CD34 are 62.14 ml, 52.95×10^7 cells, and 131×10^4 cells (Figure 1). From our data, it shows that as higher collected UCB volume, higher TNC and total Viable CD34 yielded. We analyze the correlation between time to product (≤12 hours, 12–24 hours, 24–36 hours, and 36–48 hours) and % viable CD34. Viable CD34 still stable at ≥ 94% for UCB complete processed below 36 hours and decrease into 74.04% for sample complete processed during 36–48 hours from collection time (Figure 2).
Discussion: FACT Net Cord 6 Ed. already specify the requirements for UCB unit stored for clinical administration are TNC $\geq 50 \times 10^7$ cells, viable CD34 $\geq 125 \times 10^4$ cells, and viability of CD34 cells shall be ≥85%. According to the standard, UCB quality from Indonesian population already meets the international standards criteria for clinical administrations. But for the therapy purpose which need more dosage it is recommended to collect more UCB volume to produce more TNC and Viable CD34. It is also recommended to complete stem cell processing below 36 hours, from collection time, to get >90% viable CD34 cells which more functional for cell regeneration after transplantations.
Conclusion: UCB quality from Indonesian population meets the international standards criteria for clinical applications.

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THERAPEUTIC POTENTIALS OF CORD BLOOD MONONUCLEAR CELLS TRANSPLANTATION FOR LIMB ISCHEMIA. A COMPARISON BETWEEN CD34+ AND CD34− CELLS

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Background: Cord blood -derived CD34+ cells are a well-characterized population of stem cells. Also peripheral blood mononuclear cells (MNCs) improves limb ischemia in patients with arteriosclerosis however technique of isolation CD34+ cells is highly cost in comparison to that of MNCS . Recently, CD34+ cells have also been shown to induce therapeutic angiogenesis in animal models of myocardial, peripheral, and cerebral ischemia. The mechanism by which CD34+ cells promote therapeutic angiogenesis is not completely understood, although evidence supports both direct incorporation of the cells into the expanding vasculature and paracrine secretion of angiogenic growth factors that support the developing microvasculature. Also, the mechanism of action of PBMNCs remains elusive. This work aims at determine cost benefit ratio of using three types of cell therapy in animal model of ischemia reperfusion through assay ischemic, apoptotic and fibrotic changes and how these changes will be ameliorated after cell therapy in ischemic animals.
Material and Methods: Three groups of rats were intramuscularly injected into the unilateral ischemic hindlimb by Cord blood -derived CD34+ cells, cord blood mononuclear cells (MNCs)and CD34−depleted MNCs then 7, 14 and 28 after operation samples were taken from muscle.
Methods: Age-matched rats underwent 1.5 hours of unilateral hind limb ischemia, followed by 7, 14,28 days of reperfusion .Histologic analysis of skeletal muscle fiber injury was assessed. Morphologic evidence of muscular fiber maturation was assessed by myogenin, using IHC. Markers of angiogenesis, as VEGF, and Caspase to assess apoptosis using RT PCR, at 7, 14, 28 days . Also were measured fibrosis using histochemistry and Western blots . RESULTS:, endothelial cell apoptosis and interstitial fibrosis were significantly attenuated by CD34+ cells, This study demonstrates that a low number of CD34+ cells favors reparative neovascularization and possibly myogenesis in limb ischemia, suggesting the potential use of this cell population in regenerative medicine. Lag in muscle levels of myogenin and an increased level of caspase as well as fibrosis in the rats, also these changes more ameliorated by CD34 treatement in comparison those treated by MNCs or CD34−ve cells.
Conclusion: The depletion of CD34+ cells attenuated the therapeutic effi- of MNCs in response to ischemia-induced neovascularization.

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ESTABLISHMENT OF AN AUSTRALIAN CORD BLOOD-DERIVED IPSC HAPLOBANK FOR CLINICAL USE

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Induced pluripotent stem cells (iPSC) are created by reprogramming normal human somatic cells into stem cells that can be differentiated into all cell types within the body, potentially providing a source of cells for therapeutic function. Importantly, iPSCs are immortal and therefore can be expanded and banked, providing an inexhaustible stem cell resource for both research and clinical applications. Technology is now available where Good Manufacturing Practice (GMP)—compliant, clinical grade iPSCs can be created for therapeutic use [1]. Recent publications have promulgated the possibility of global iPSC banks, in which the banked lines have homozygous human leukocyte antigen (HLA) haplotypes and are derived from material selected from donors whose haplotypes are common in the target population. As such, cells derived from these banked iPSCs would be suitable for therapeutic use for many individuals within the population. Cord blood (CB) is an ideal source of starting cells for iPSC generation.
We aim to establish a clinically relevant GMP compliant bank of homozygous HLA haploidentical cord blood derived hiPSC lines for cellular therapies in Australia and globally. In a collaboration between the iPSC core facility at MCRI, the BMDI Cord Blood Bank (BMDI CBB) and Sydney Cord Blood Bank (SCBB), experts in HLA and statistical genomics and experts in GMP and international regulatory compliance, we have embarked upon a project to establish the infrastructure and explore the feasibility of such a bank. HLA tissue typing data from CB banked at the BMDI CBB was interrogated using a purpose-written algorithm; from a total of 13,679 records interrogated at least 143 CBU with homozygous haplotypes at the 2-digit level of HLA-A, B and DRB1 were identified, with at least 10–20 CBU with unique homozygous haplotypes. 17,526 records were interrogated from the SCBB, with at least 10 CBU with unique homozygous haplotypes identified. This preliminary data confirms the feasibility of the establishment of an Australian Cord Blood iPSC haplobank for clinical use. Progress towards this aim will be presented, highlighting the many challenges and considerations ahead.

Reference

[1] Baghbaderani BA, Tian X, Neo BH, Burkall A, Dimezzo T, Sierra G, et al. cGMP-manufactured human induced pluripotent stem cells are available for pre-clinical and clinical applications. Stem Cell Reports 2015;5(4):647–59.

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DIFFERENTIAL EFFECT OF PROGNOSTIC FACTORS IN HEALTH AND DISEASE IN PERIPHERAL BLOOD STEM CELL (PBSC) MOBILIZATION AND COLLECTION: A SINGLE-CENTER/DOCTOR EXPERIENCE IN AUTOLOGOUS HARVEST

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Background and Objectives: Granulocyte–Colony Stimulating Factor(G-CSF) stimulated peripheral blood stem cell collection (PBSC) has effectively replaced bone marrow harvest as a safe stem cell source for autologous transplantation in the past 20 years. Poor collection outcomes are still common in different populations and this study adds important prognostic factor that may affect outcomes.
Material and Methods: PBSC was collected after mobilization with 5 µg/Kg SC daily for 5 days in 216 healthy donors and 131 patients [median donor age at 41 (15–74) years while patient median age was 52 (7–89)] using 2 types of continuous blood cell separator (COBE Spectra or CombTec) on day 6 for healthy donors while some patients needed additional PBSC collection on day 7. Total cell yields were calculated as the number of CD34+ cells/kg body weight (BW). The efficacy PBSC collection compare between day 1 and 2 by using pair T-test. All subjects were monitored for PBSC collection side effects such as hypocemia and platelets loss.

Background:

The therapeutic potential of cord blood grow over the last few days .

Regenerative medicine is the science of which the living cells being used to facilitate repair tissue damage (genetic, injury or aging).

In young patients it change the untreatable condition as autism.

Regenerative medicine promises some extraordinary medical possibility.

Today it can be use for:

Burns, liver diseases, heart disease, peripheral limb ischemia, autism, brain injury, multiple sclerosis, cerebral palsy, stroke, Parkinson's disease, heart attack, bone fracture, DM.

Source of regenerative 'stem' or progenitor cells—for example, hematopoietic progenitor cells (HPCs) and mesenchymal stem cells (MSCs)—for use against many .human diseases (Santiago etal, 2015)

Stem cells are also considered to be a cornerstone of new area of science known as regenerative medicine.

In the near future , stem cells will provide cure for common and life threating conditions : heart disease, Alzheimer's and DM.

Types of stem cells:

Broadly speaking, the main types of stem cells, embryonic and non-embryonic.

Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst and can differentiate into cells of all three germ layers.

However teratoma formation and ethical controversy hamper their research and clinical application

On the other hand, non-embryonic stem cells, mostly ***adult stem cells,*** *are already somewhat specialized and have limited differentiation potential. They can be isolated from various tissues and are currently the most commonly used seed cells in regenerative medicine.*

*Recently, another type of non-embryonic stem cells, known as induced pluripotent stem cell (**iPSCs**) has emerged as a major breakthrough in regenerative biology. They are generated through enforced expression of defined transcription factors, which reset the fate of somatic cells to an embryonic stem-cell-like state*

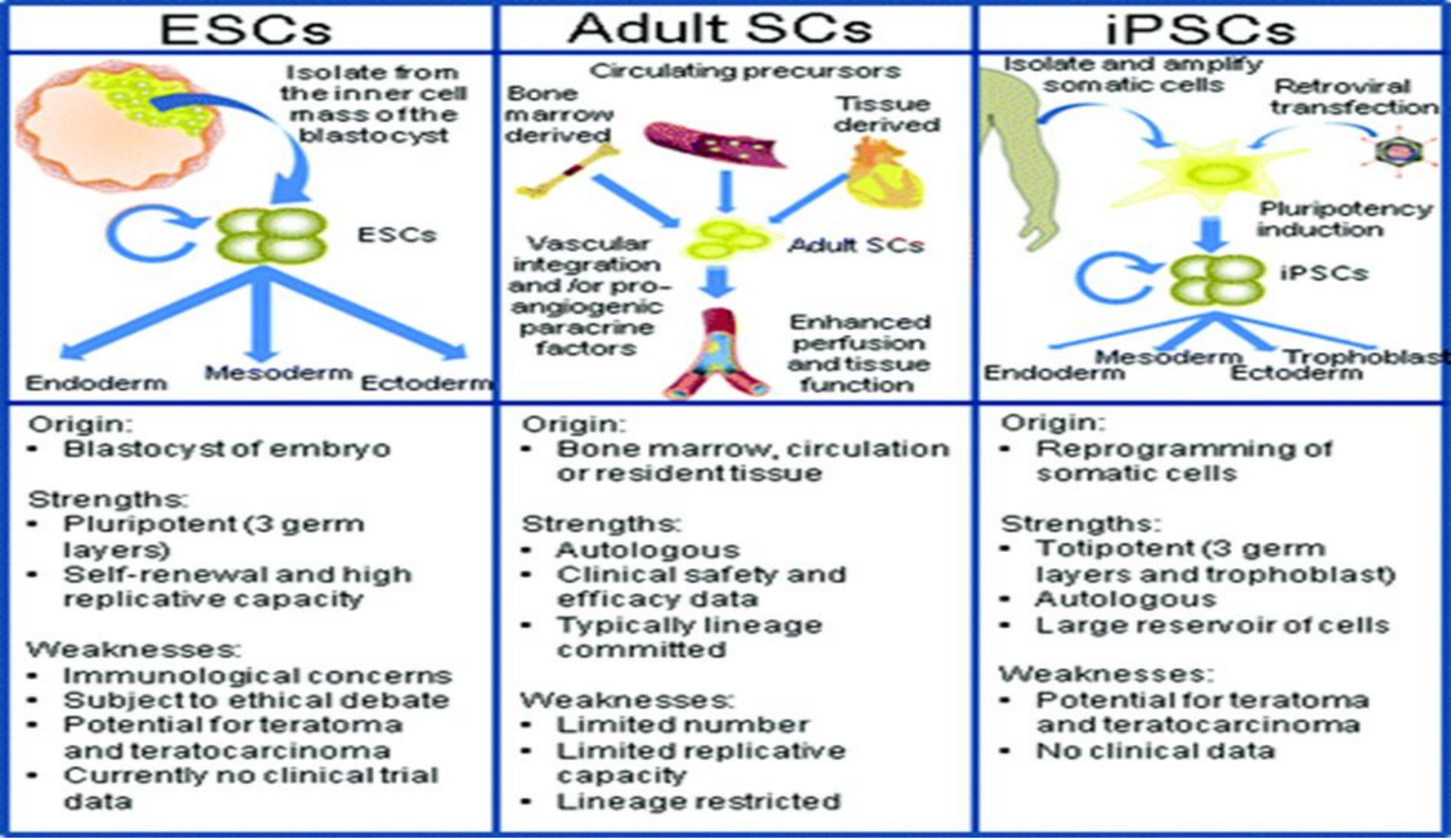


Fig (1): Types of Stem Cells , ESCs embryonic stem cells, iPSCs induced pluripotent stem cells. Leeper et al,(2010), Circulation. 2010;122:517-526

Cord blood stem cells are a precious resource that have been saving lives for over 20 years. There have been more than 1 million stem cells treatments worldwide.

The Umbilical cord blood stem cells are the recognized therapy for 80 diseases.

These include:

***Blood disease**: aplastic anemia, FA, leukemia, lymphoma.*

***Cancer**: solid tumor: neuroblastoma*

***Immune disorder** : SCID, Wiskott-Aldrich syndrome.*

Inborn error metabolism: Gucheur.

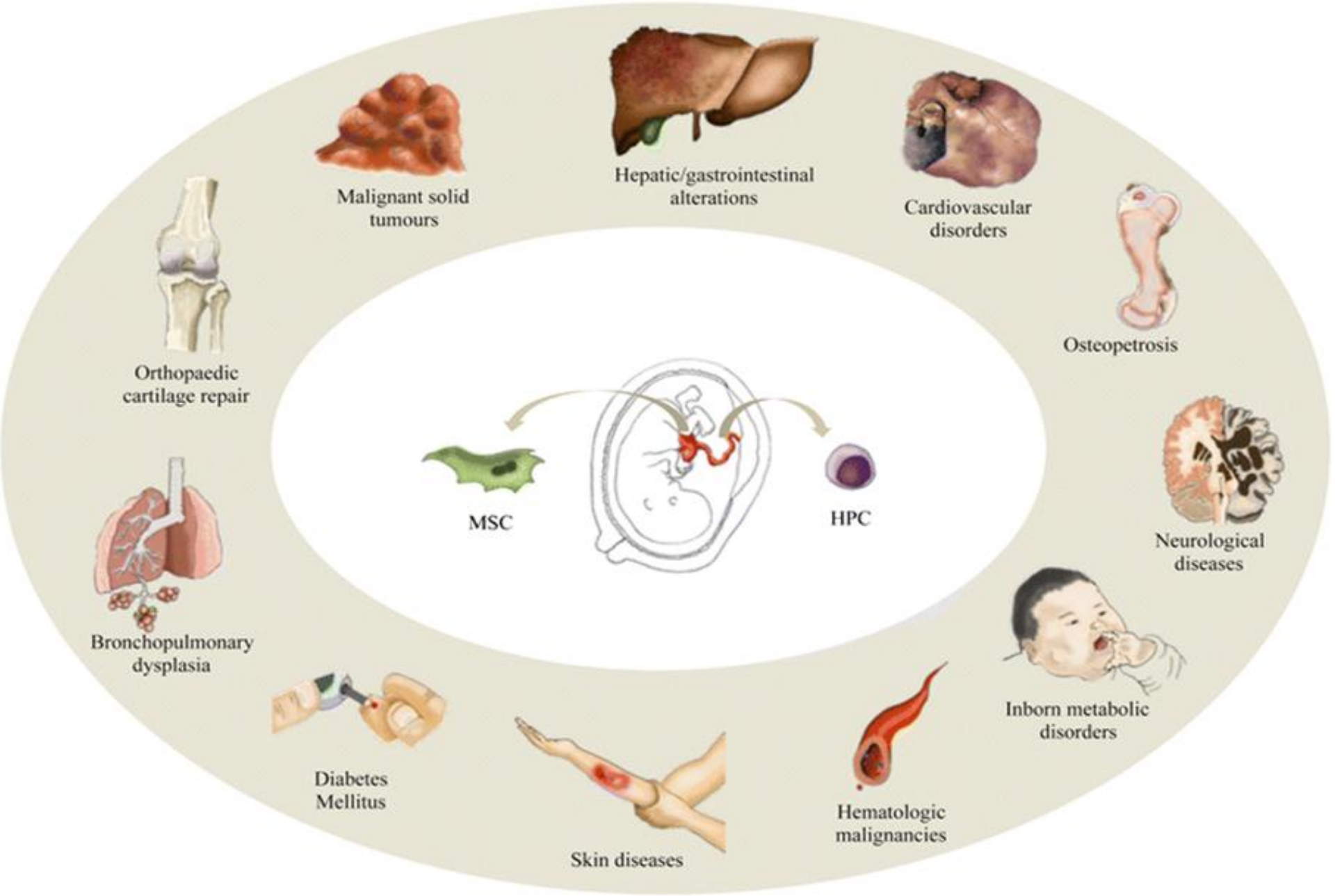
C.N.S: Myopathy, neurodegenerative disease, cerebral palsy. Autism, Spinal cord injury

Heart disease, MI, PLI.

Umbilical cord blood *contains millions of hematopoietic stem cells (HPSCs).*

These type of stem cells can transform to any type of blood cells.

So cord blood can be to rebuild the immune system in patients with advanced cancer whom taken chemotherapy.



- **Fig (2): Current clinical applications of umbilical cord blood**. Stem Cell Research & Therapy2015 **6** :123

Cord blood registry : CBR 1992

south California

regulated clinical trials approved by FDA

about 500,000 families are members used the baby cord blood.

Accredited by AABB American Association of Blood Banking .

They use seamless cryo bag to avoid breakige

When you bank your child(baby) cord blood?

1- Autologous stem cell transplantations

2- Sibling , more suitable than allogeneic transplantation.

Cord blood *-derived CD34^{+} cells are a well-characterized population of stem cells. Also mononuclear cells (MNCs) improves limb ischemia in patients with arteriosclerosis .*

*(Santiago et al, 2015) Stem Cell Research & Therapy2015**6**:123*

Transplantation of HPSCs CD34+ cells from cord blood needs the presence of MSCs for expansion in cytotherapy , feeder layer decrease apoptosis (Roya Mehrasa etal, 2014)

*Critical limb ischemia (CLI) represents the most advanced stage of peripheral arterial disease (PAD) with a markedly reduces blood flow to the extremities and has progressed to the point of severe rest pain Critical limb ischemia (CLI) is an important condition in the general population with a strong social impact and/or even tissue loss (*Strauss B. H,2013).

Recent therapeutic strategies have focused on restoring this tissue survival using exogenous cellular agents to promote regeneration of the vasculature. These are based on stimulation of angiogenesis

Intramuscular and intra-arterial injection or a combination of both may be proposed in the treatment of human PAD. The principle of intramuscular injection is the creation of a cell depot with paracrine activity in the ischemic area (Liao etal,2013).

Injection of bone marrow mononuclear cells has been reported to promote neovascularization of ischemic tissues effectively. This angiogenic effect may be related to their ability to induce vascular and muscle regeneration by paracrine mechanisms through vascular endothelial growth factor secretion. Bone marrow mononuclear cells (BM-MNCs) release various angiogenic factors (Rita etal, 2015).

FCM markers for stem cells:

1-Hematopoietic stem cell (HPSCs)

isolated from P.Bl, B.M or umbilical cord .

Positive for CD34+ve , CD45+ve

2-MSCs : isolated from adipose tissue, placenta, umbilical cord , B.M and tooth bulb .

Negative for CD34, CD45, CD19, CD3, CD11b, HLA-DR

Positive for CD90, CD271 CD71, CD105,CD73, CD44

However technique of isolation $CD34^+$ cells is highly cost in comparison to that of MNCS. Recently, $CD34^+$ cells have also been shown to induce therapeutic angiogenesis in animal models of myocardial, peripheral, and cerebral ischemia. (Gopall et al, 2010).Cite this article as: BJMP 2010;3(4):a345

The mechanism by which $CD34^+$ cells promote therapeutic angiogenesis is not completely understood, although evidence supports both direct incorporation of the cells into the expanding vasculature and paracrine secretion of angiogenic growth factors that support the developing microvasculature. (Grande et al, 2015) Stem Cell Int.

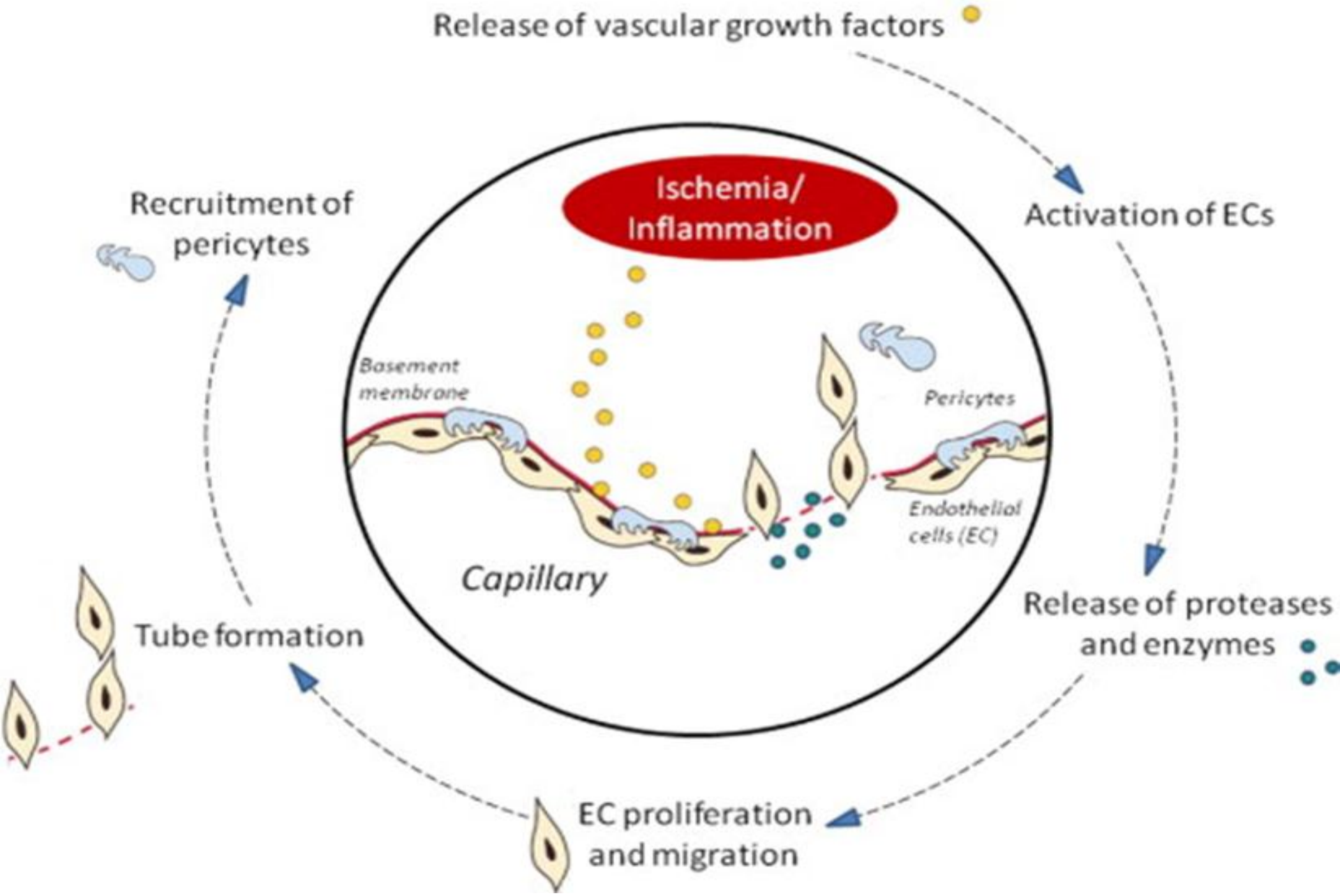
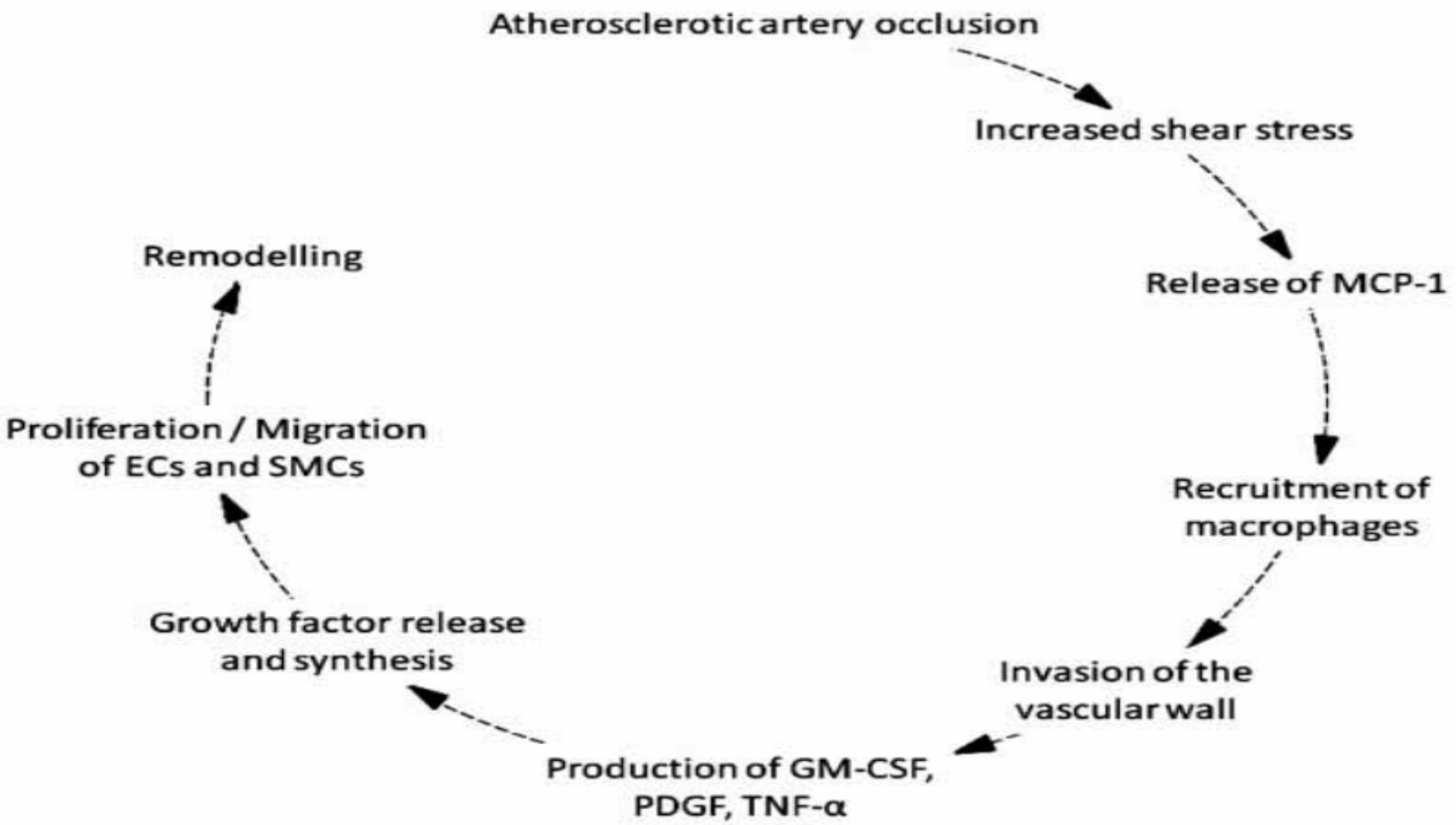


Figure 3: Schem of possible events in the angiogenic process. EC, endothelial cell.
(**Lawall et al. Autologous stem cell therapy** 2010) Thrombosis and Haemostasis 103.4/2010



Fig(4): ***Schema of angiogenesis events .*** ***(****EC: endothelial cell; SMC: smooth muscle cell; MCP-1: monocyte chemoattractant protein-1; GM- CSF: granulocyte -macrophage colony-stimulating factor; PDGF platelet derived growth factor; TNFα, tumour necrosis factor-α. (* ***Lawall et al. Autologous stem cell therapy*** *2010) Thrombosis and Haemostasis 103.4/2010*

Aim:

To study benefit of three types of cell therapy in limb ischemia / reperfusion model through assay ischemic, apoptotic, fibrotic and angiogenesis events before and after cell therapy .

METHODS :

- *The study was done at Molecular Biology and Cell Culture Unit of Assiut University.*
- *During the period from May 2015 to May 2017*
- *It was approved by institutional research committee.*

Methods :

Three groups of age matched rats were subjected to unilateral hind limb ischemia for 1.5 hours, serum myoglobin, CK were assayed, 3 hours after reperfusion

- *Cord blood CD34+ cells , MNCs, and depleted CD34 cells were isolated by Ficoll™ , polysaccharide based density for MNCs , magnetic probe for CD34+.*
- *Identified by BD FCM eight color (USA) using monoclonal Abs to CD34 .*
- *Expanded on DEMEM, cryopreserved till use*

- *Intramuscular injection by cord blood* ***CD34^{+}*** *cells, cord blood* ***(MNCs)*** *and* ***CD34 depleted*** *MNCs*
- *Then 7, 14 and 28 days after operation muscle biopsy were taken , Caspase, HLA- ABC, VEGF, using RT-PCR, myogenin using IHC*

- *The number of injected concentrated mononuclear cells (MNCs) in PAD between $0.3x10^9$ to 2×10^9 MNCs*

  The percentage of implanted CD34+ cells is usually between 0.6% and 2.4% of total implanted MNC .

- *(2.5X10 6 CD 34 +) increase therapeutic effect. Associated with fever , post transfusion syndrome, decrease hematopoietic reserve*

Results :

Endothelial cell apoptosis and interstitial fibrosis were significantly attenuated by cord blood CD34+ cells, and (MNCs), the depletion of CD34 attenuates the therapeutic efficiency of MNCs induced neovascularization

Items	ANG2	Caspase	VEGF	GAPDH
One week				
CD34+ve	31.62±2.94	33.78±3.14	19.73±1.84	25.42±2.36
CD34-ve	9.83±0.91	36.95±3.44	20.81±1.94	31.54±2.93
ISC	7.79±0.72	19.9±1.85	20.19±1.88	27.71±2.58
Mono	32.55±3.03	36.39±3.38	21.22±1.97	20.98±1.95
Two weeks				
CD34+ve	31.46±2.93	35.97±3.34	20.28±1.89	13.15±2.71
CD34-ve	6.32±0.59	29.9±2.78	19.96±1.86	30.3±2.82
ISC	19.4±1.8	24.84±2.31	19.95±1.86	33.43±1.25
Mono	33.5±3.12	36.17±3.36	21.49±2	17.04±2.51
One month				
CD34+ve	31.81±2.96	37.21±3.46	21.57±2.01	13±3.07
Control	7.02±0.65	26.92±2.5	19.69±1.83	33.87±1.29

	ANG2	GAPDH	P. value	Fold change
One week				
CD34+ve	31.62±2.94	25.42±2.36	<0.001**	0.00024
CD34-ve	9.83±0.91	31.54±2.93	<0.001**	59491.5
ISC	7.79±0.72	27.71±2.58	<0.001**	17312.4
Mono	32.55±3.03	20.98±1.95	<0.001**	0.00001
Two weeks				
CD34+ve	31.46±2.93	13.15±2.71	0.311	0.00351
CD34-ve	6.32±0.59	30.3±2.82	<0.001**	288574.6
ISC	19.4±1.8	33.43±1.25	<0.001**	0.00028
Mono	33.5±3.12	17.04±2.51	<0.001**	0.00020
One month				
CD34+ve	31.81±2.96	13±3.07	0.214	0.03953
Control	7.02±0.65	33.87±1.29	<0.001**	

	Caspase	GAPDH	P. value	Fold change
One week				
CD34+ve	33.78±3.14	25.42±2.36	<0.001**	51.8
CD34-ve	36.95±3.44	31.54±2.93	0.012*	400.6
ISC	19.9±1.85	27.71±2.58	<0.001**	383588.6
Mono	36.39±3.38	20.98±1.95	<0.001**	0.39269
Two weeks				
CD34+ve	35.97±3.34	13.15±2.71	<0.001**	151.3
CD34-ve	29.9±2.78	30.3±2.82	0.719	22551.8
ISC	24.84±2.31	33.43±1.25	<0.001**	6.3
Mono	36.17±3.36	17.04±2.51	<0.001**	30.3
One month				
CD34+ve	37.21±3.46	13±3.07	0.047*	919.4
Control	26.92±2.5	33.87±1.29	<0.001**	

	VEGF	GAPDH	P. value	Fold change
One week				
CD34+ve	19.73±1.84	25.42±2.36	<0.001**	5837.3
CD34-ve	20.81±1.94	31.54±2.93	<0.001**	192081.6
ISC	20.19±1.88	27.71±2.58	<0.001**	20833.1
Mono	21.22±1.97	20.98±1.95	0.428	96.1
Two weeks				
CD34+ve	20.28±1.89	13.15±2.71	<0.001**	53038.8
CD34-ve	19.96±1.86	30.3±2.82	<0.001**	146801.4
ISC	19.95±1.86	33.43±1.25	<0.001**	1.23029
Mono	21.49±2	17.04±2.51	<0.001**	5310.1
One month				
CD34+ve	21.57±2.01	13±3.07	<0.001**	311970.5
Control	19.69±1.83	33.87±1.29	<0.001**	

Conclusion:

- *Endothelial cell apoptosis and interstitial fibrosis were significantly attenuated by cord blood CD34+ cells, and (MNCs)*
- *Depletion of CD34 attenuates the therapeutic efficiency of MNCs induced neovascularization*

Cord blood MNCs can be used in regenerative therapy of limb ischemia instead of isolation of CD34 as cost benefit rational .

Take Home Message:

- *Limb ischemia is critical condition lead to amputation if no interference*
- *CD34+ cells is highly effective , with high cost*
- *CD34- cells has a minimum role on therapy*
- *Cord blood MNCs can give therapeutic efficacy as much as CD34+ cells*


Thank You