CORD BLOOD STEM CELLS FOR CARDIO VASCULAR DISEASE

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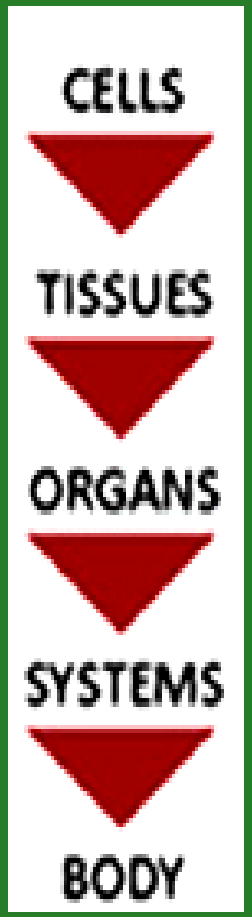
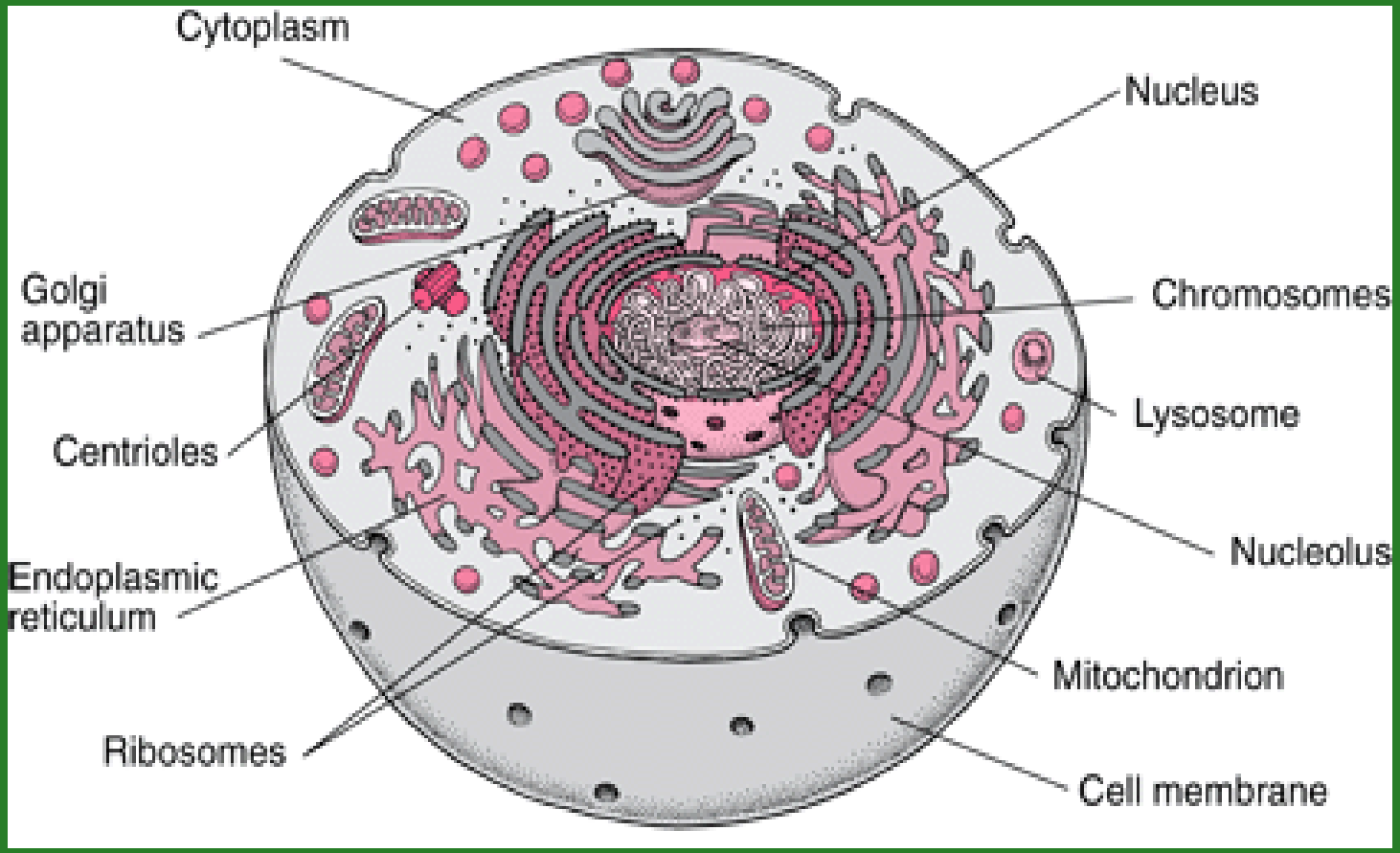
WCC&IVUS2015

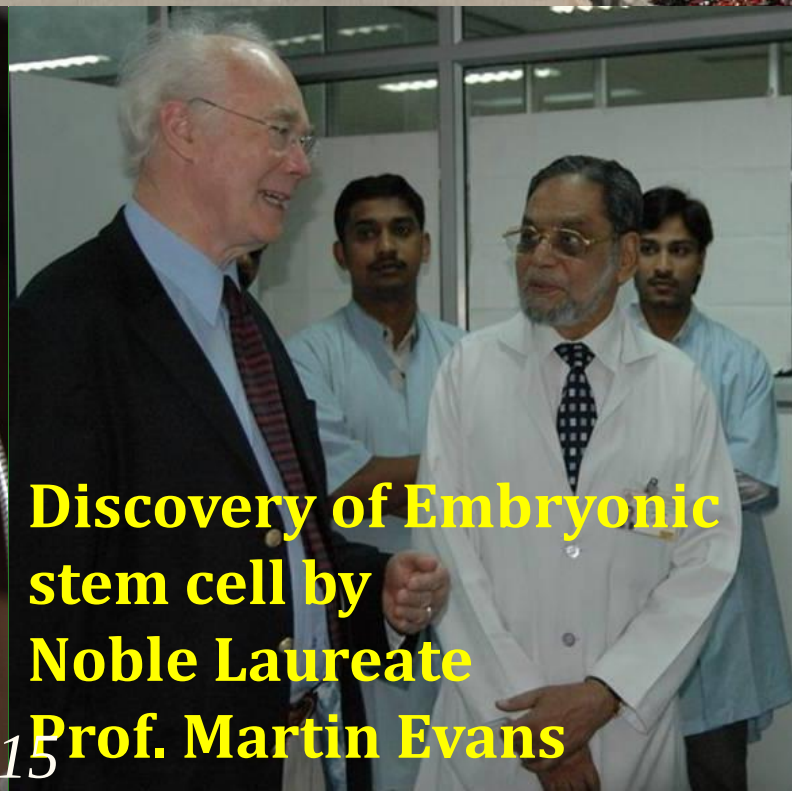
Cell

(structural & functional unit of life)





1st Cell-based therapy performed at Osmania Hospital, Hyderabad By
Prof. C. M. Habibullah



Discovery of Embryonic stem cell by Noble Laureate Prof. Martin Evans

Cardiovascular disease (CVD)

- A leading cause of death worldwide.
- According to the WHO:

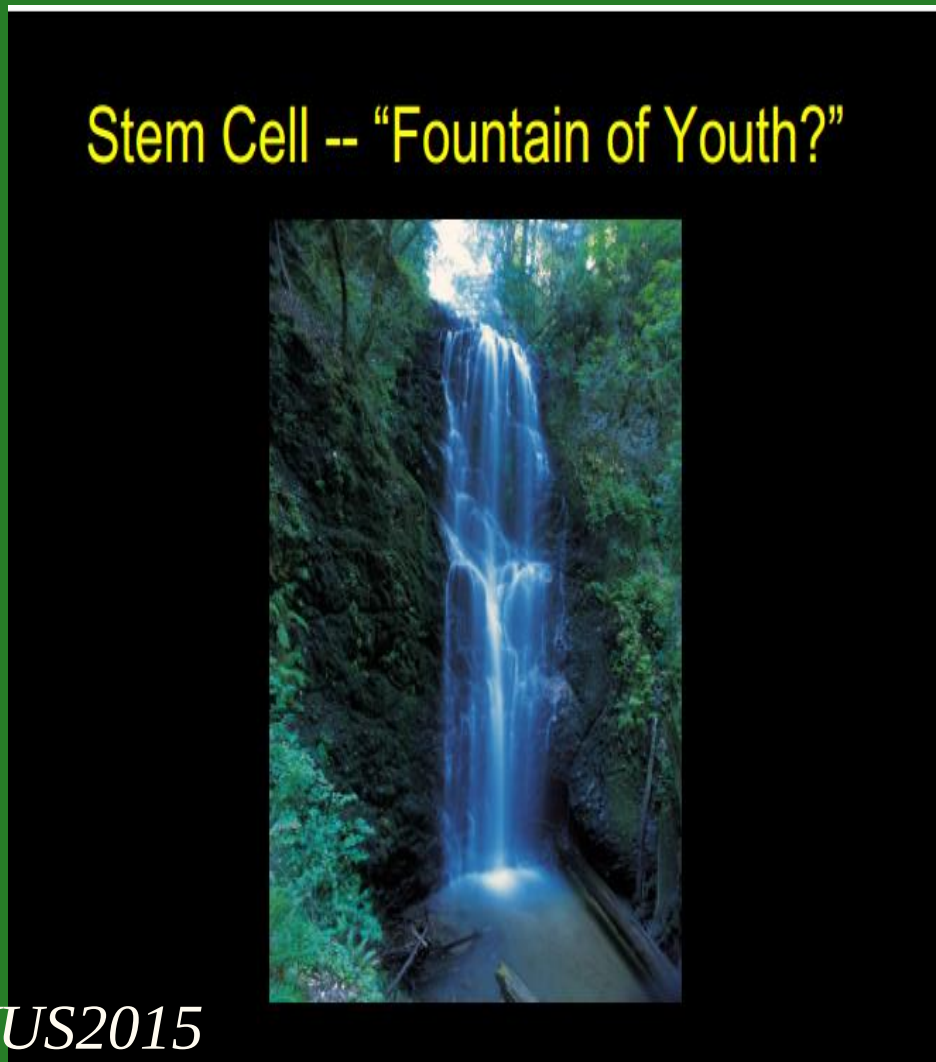
  an estimate of 17.3 million people died from CVDs in 2008 and by 2030, the number of deaths is estimated to reach almost 23.6million.
- complicated by the limited ability of the heart for self-regeneration
- Despite, the development of a wide array of treatment options, heart failure management has failed to replace the lost cardiomyocyte mass with new contractile cells

Cont…

- Myocardial infarction (MI, aka heart attack) occurs as a result of cardiomyocytes death leading to loss of viable myocytes, which lack endogenous repair mechanisms.
- If left untreated, it will lead to fibrous scar formation replacing the damaged myocardium with subsequent congestive heart failure
- This led to the introduction of cell-based therapeutic approaches to treat the damaged heart

Novel Therapies Are Much Needed

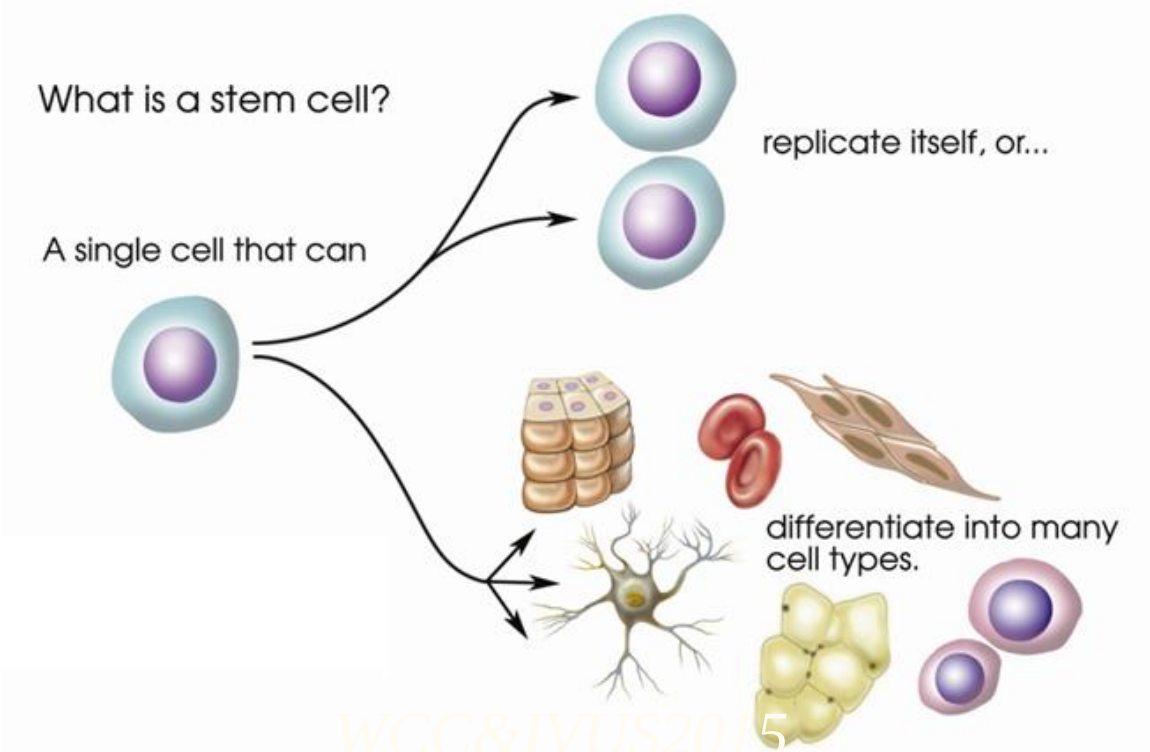
- Emergence of **Stem cell concept** in 2000 for cell-based therapies has become a very active area of research
- This has lead to create a new era of regenerative medicine



FACT vs. FICTION

STEM CELLS

WHAT | WHERE | HOW



Stem Cell Therapy in Cardiac Diseases

Clinical studies have focused on 3 main situations

- Acute MI (with the hope of preventing LVSD)
- Chronic heart failure secondary to previous MI
- DCM (non ischemic cardiomyopthy)

Recent clinical studies using drugs

Trial	Year/ reference	Study design	Patients (number)	Patients (diagnosis)	Drug type	Drug dose/ application	Follow up (months)	Follow up (method)	Functional outcome (LVEF)
EPOC-AMI	2010 (18)	r, ol, be, c	35	STEMI (PCI)	EPO (low dose)	One dose of 6,000 IU during PCI	6	SPECT	Significantly improved by 6.5% (EPO group baseline *vs.* 6 months follow-up, P=0.003), no significant improvement in controls
Bergmann *et al.*	2011 (19)	r, db, pc	28	HF (PCI)	EPO (low dose)	35 IU/kg body weight (weekly for 6 months)	6	echo, MRI	Significantly improved by 4.9% (echo, P=0.019) and 5.0% (MRI, P=0.042) (EPO group *vs.* placebo)
STEM-AMI	2010 (20)	p, r, sb, pc	60	STEMI (PCI) (LVEF <45%)	G-CSF	G-CSF 5 μg/kg subcutaneously (daily for 5 days)	6	MRI	No significant improvement (G-CSF group *vs.* placebo)
MAGIC Cell-3-DES	2012 (21)	r, c	117	MI (PCI)	G-CSF + subsequent injection of PBMCs	G-CSF 10 μg/kg subcutaneously (daily for 3 days) + PBMC injection (IC)	24	MRI	No significant improvement (G-CSF/PBMC group *vs.* control)

Recent clinical studies using different types of stem cells

Trial	Year/ reference	Study design	Patients (number)	Patients (diagnosis)	Cell type	Application	Follow up (months)	Follow up (method)	Functional outcome (LVEF)
BOOST	2009 (10)	r, c	60	STEMI (PCI)	BMCs	ic	~60	MRI	No significant improvement (BMC group vs. control)
REPAIR-AMI	2010 (11)	r, db, pc	204	STEMI (PCI) (LVEF <45%)	BMCs	ic	24	MRI (only 59 patients)	No significant improvement (BMC group vs. control)
TOPCARE-AMI	2011 (12)	r	55	STEMI (PCI)	CPCs or BMCs	ic	60	MRI	Improvement by 11% (BMC/CPC group: baseline vs. 5-year follow up, P<0.001), no control group
C-CURE	2013 (2)	r, c	48	CHF (LVEF 15-40%)	CPSCs (derived from MSCs)	ic	6	echo	Improvement by 7% (CPSC group: baseline vs. 6 months follow up, P<0.0001), no change for control group

Cont…

CELLWAVE	2013 (13)	r, pc	103	CHF post MI (LVEF <50%)	Shock wave pretreatment + BMCs	ic	4	LVA	Improvement by 3.2% (shock wave + BMC), improvement by 1% (shock wave + placebo) (P=0.02)
APOLLO	2012 (14)	r, db, pc	14	STEMI (LVEF 30-50%)	ADRCs	ic	6	SPECT	No significant improvement (ADRC group vs. placebo)
CADUCEUS	2012 (15)	r, c	25	Recent MI (LVEF <45%)	CDCs	ic	6,12	MRI	LVEF: no difference
SCIPIO	2012 (16,17)	r, c	33	MI (CABG) (LVEF <40%)	CDCs (c-kit+)	ic	4,12	MRI	Improvement by 7.6% (CDC group baseline vs. 4 months follow up; P=0.004, n=8); improvement by 13.7% (CDC group baseline vs. 4 months follow up:

No consistent results with these clinical studies

Potential mechanisms of stem cells action in the diseased heart

- Trans-differentiation of stem cells into cardiomyocytes
- Induced growth of resident cardiomyocytes by paracrine effects
- Stimulation of resident endogenous myocardial stem cells
- Cell fusion between transplanted cells and resident cardiomyocytes

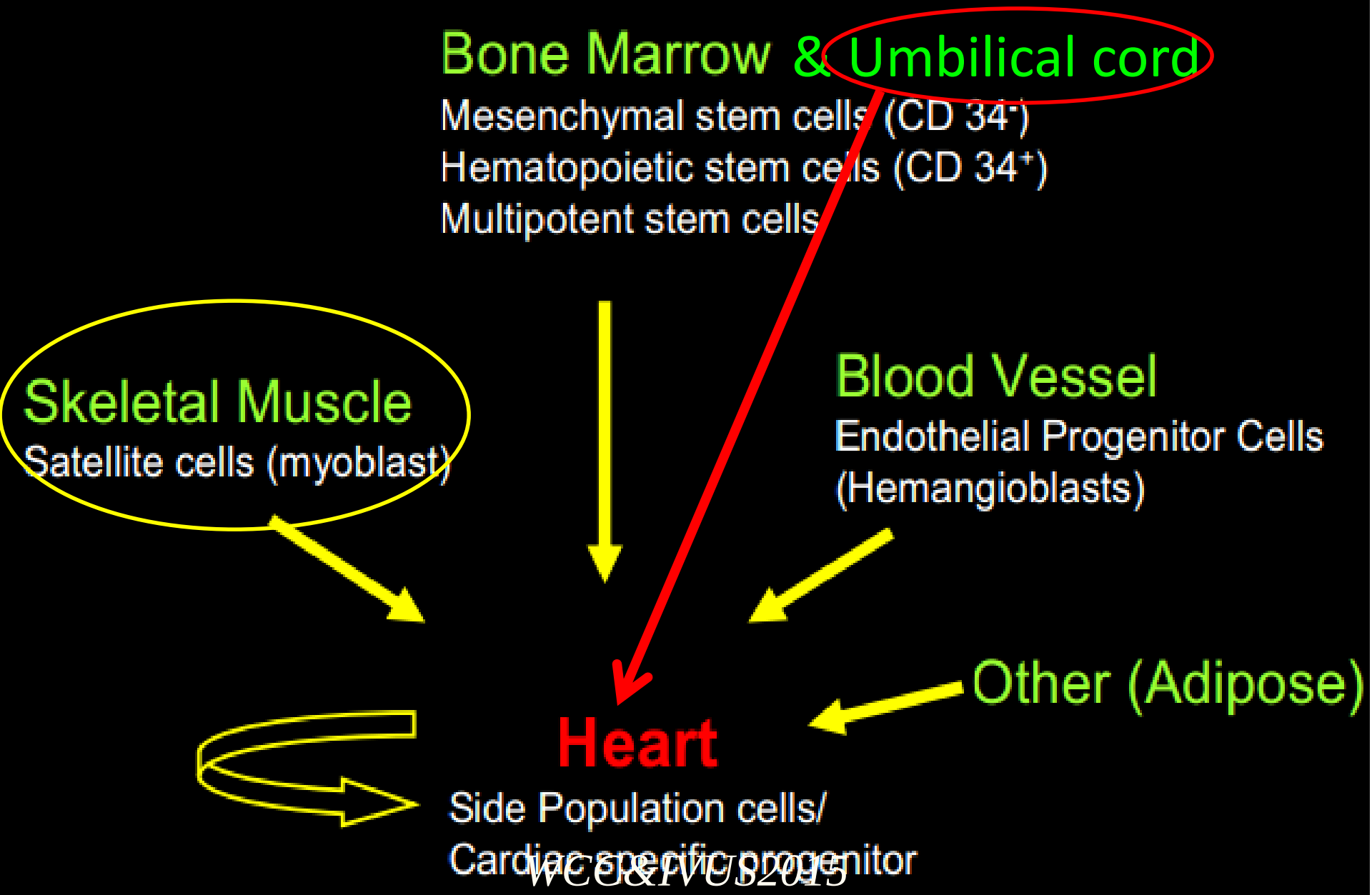
Types of Stem Cells

1. **Embryonic stem cells (ESCs)**
2. **Adult stem cells (ASCs)**
   - Hematopoietic stem cells (HSCs),
   - Mesenchymal stem cells (MSCs),
   - Endothelial progenitor cells (EPCs),
   - Cardiac progenitor cells,
   - Skeletal myoblasts,
   - Induced pluripotent stem cells (iPSCs)

Embryonic stem cells

- ESCs are the prototypical stem cells
- ESCs can differentiate into any cell present in the adult organism and have the potential to completely regenerate the myocardium
- **Two major limitations that** stand in the way of the therapeutic use of ES cells are:
  - Immunological rejection &
  - the propensity of ES cells to form teratomas when injected in vivo

Adult Stem Cell Therapy and the Heart



Skeletal myoblasts

- Skeletal myoblasts are ideal cells to regenerate myocardium
- Myoblasts are resistant to ischaemia, can differentiate into myotubes in *vivo* and improve ventricular function
- Experimental studies have demostrated its safety and efficacy of skeletal myoblasts in animal models

Endothelial progenitor cells (EPCs)

- EPCs are a subset of haematopoietic cells having potential to differentiate into endothelial cells
- EPCs have a role in promoting angiogenesis
- EPCs also provides paracrine survival signals to cardiomyocytes
- EPCs are readily isolated from the blood and the bone marrow, and clinical studies suggest that cell-based therapy with EPCs can improve myocardial function

Hematopoietic stem cells

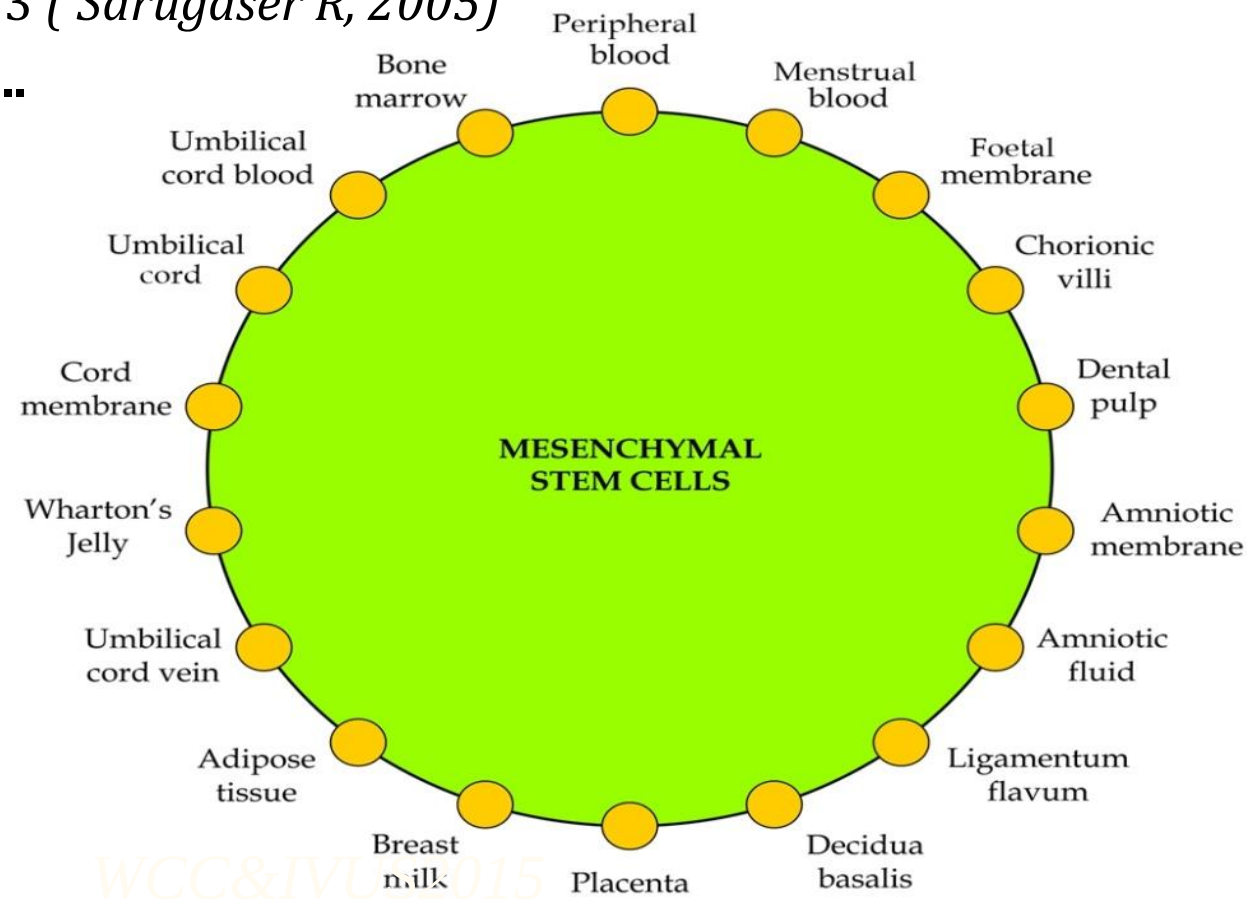
- Initial studies have demonstrated use of HSCs in cardiac regeneration
- Animal studies of bone-marrow transplantation with labeled HSCs followed by myocardial infarction revealed cardiomyocytes derived from the transplanted cells, but at an exceptionally low rate
- Differentiation of HSCs to cardiomyocytes is still experimental ?

Mesenchymal stem cells (MSCs)

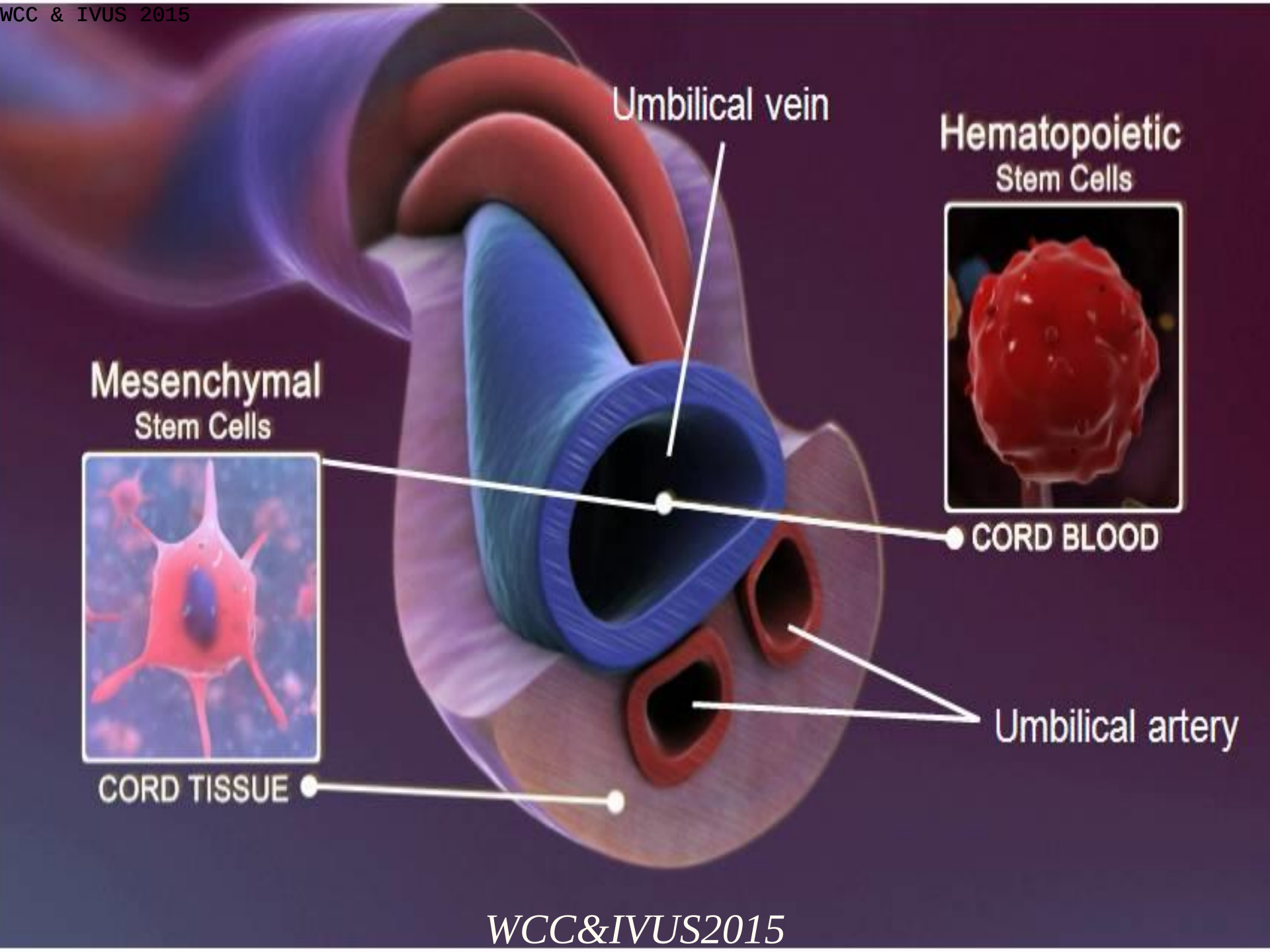
- Multipotent, plastic adherent, fibroblast-like cells
- Able to differentiate into mesodermal lineage, and trans differentiate to ectodermal and endodermal cell types
- Secrete growth factors, cytokines and chemokines with anti-inflammatory, angiogenic properties
- Non-tumorigenic in nature
- Home/migrate to damaged/inflamed tissues
- Hypoimmunogenic in nature and can inhibit immune responses

Sources of MSCs isolation

- **Bone Marrow:** 1 in 10^5 *(Beresdorf JN, 1989*)
- **Adipose tissue:** 2 in 100 *(Fraser JK, 2004)*
- **Cord blood:** 0-2 in 10^8 *(Bieback K, 2004)*
- **Wharton's Jelly:** 1 in 333 *(Sarugaser R, 2005)*
- **And many more……**

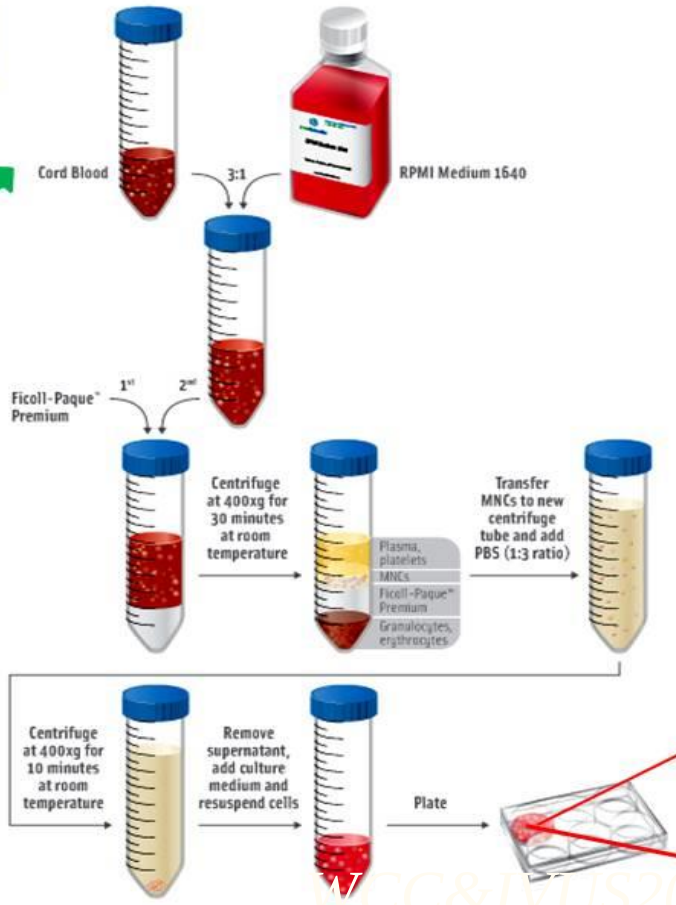


Umbilical cord blood (UCB): A most appropriate source for MSCs

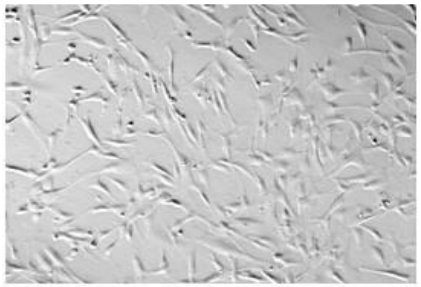

Umbilical vein
Hematopoietic
Stem Cells
Mesenchymal
Stem Cells
CORD BLOOD
Umbilical artery
CORD TISSUE

Isolation and in vitro culture of MSCs from UCB

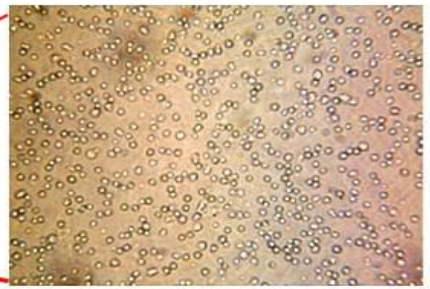
Ficoll-paque density gradient separation



Mesenchymal stem cells

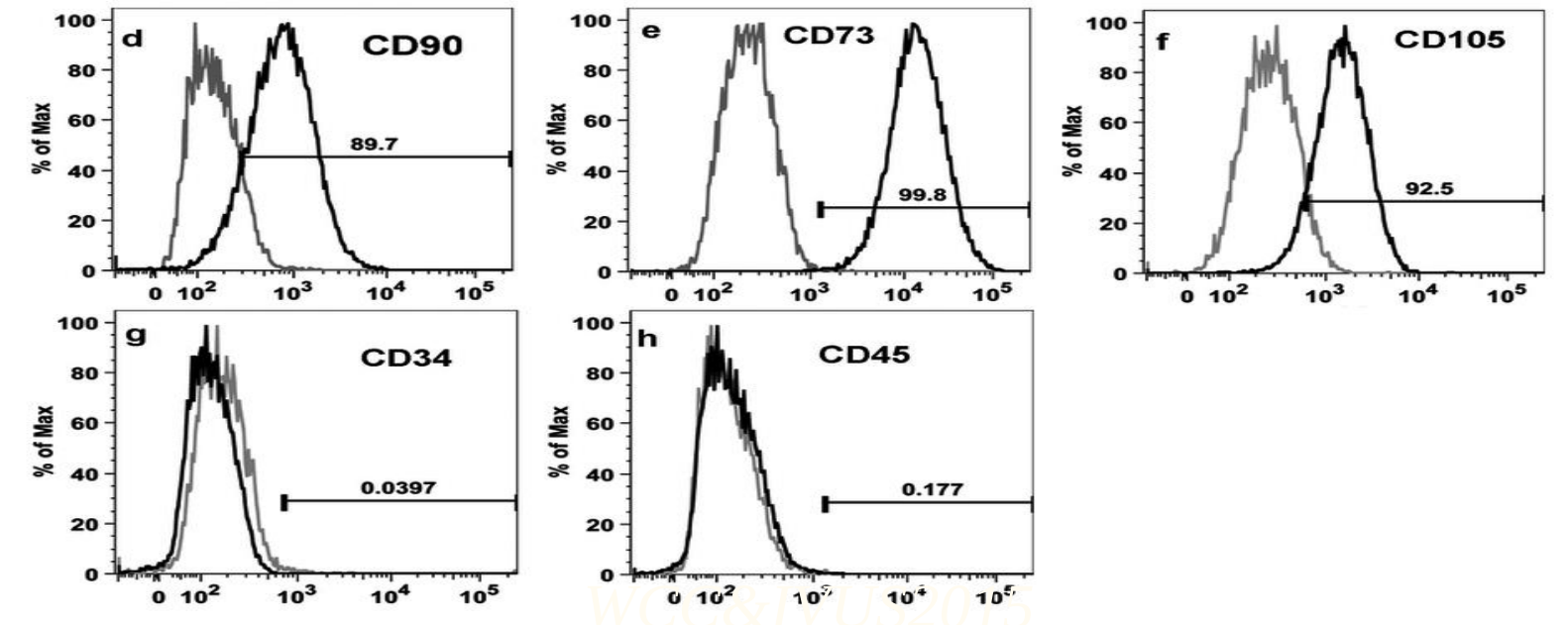


After 7-10 days



MSCs characterization

Markers (+)	Markers (-)
CD90	CD34
CD105	CD45
CD73	CD14



In vitro differentiation of UCB mesenchymal stem cells to cardiomyocyte lineage

- Can be differentiated using 5-Azacytidine supplemented with growth factors (Wu et al. 2009)
- Morhological appearance: Flattened
- Expresses: cardiac actin, sarcomeric actinin, connexin 43

Challenge

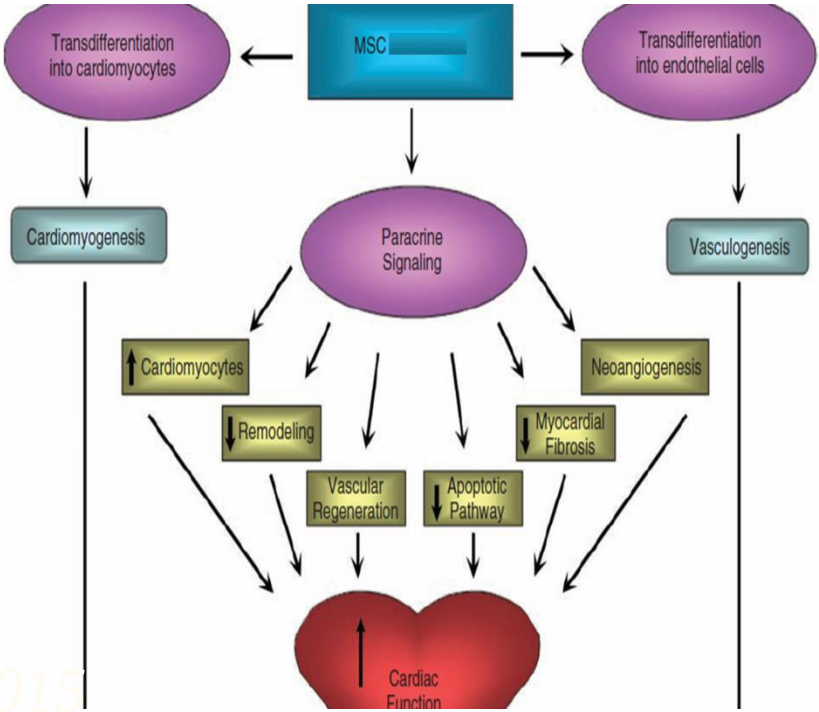
- Shows less expression of Tropinin and myosins expression which is necessary for contraction

Potential of UCB-MSCs in Cardiac Regeneration

Paracrine effect

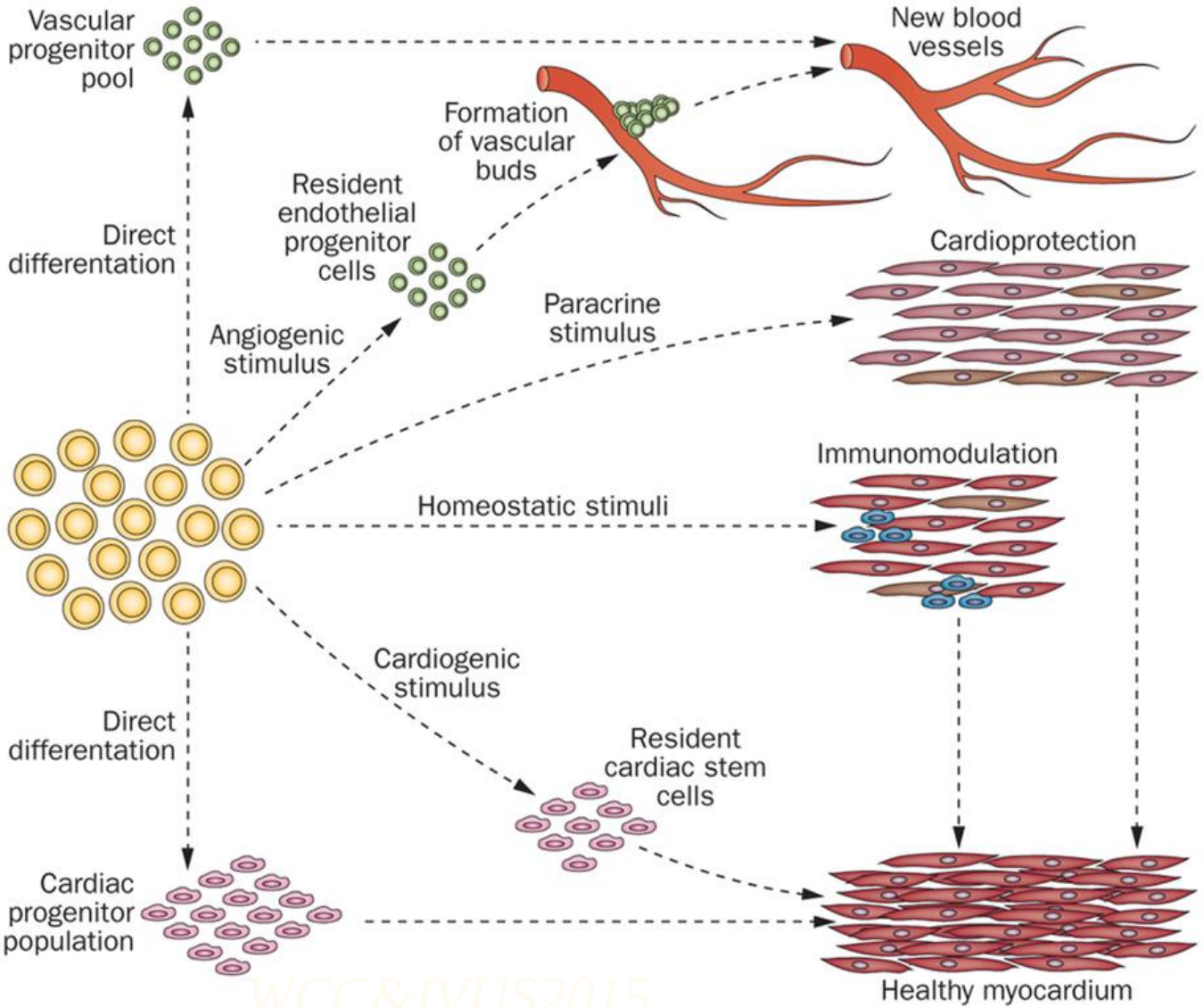
Vascular regeneration

Cardiomyocyte regeneration



Therapeutic mechanism of UCB-MSCs in cardiac regeneration

UCB-MSCs



Translational applications of UCB-MSCs: Bench to bedside: the optimal milieu for cells to thrive?

- Selection of appropriate cell type
- Generation of cardiomyocyte
- Proliferation
- Integration
- Synchronized function

A new approach for the development of humanized bio-artificial Heart using xenogenic organ scaffold

New strategies needs 5 major components for recreation/reconstruction of tissues and whole organs

Three dimensional natural bioscaffolds

Intact native microvasculature

Intact native ECM (to certain extent)

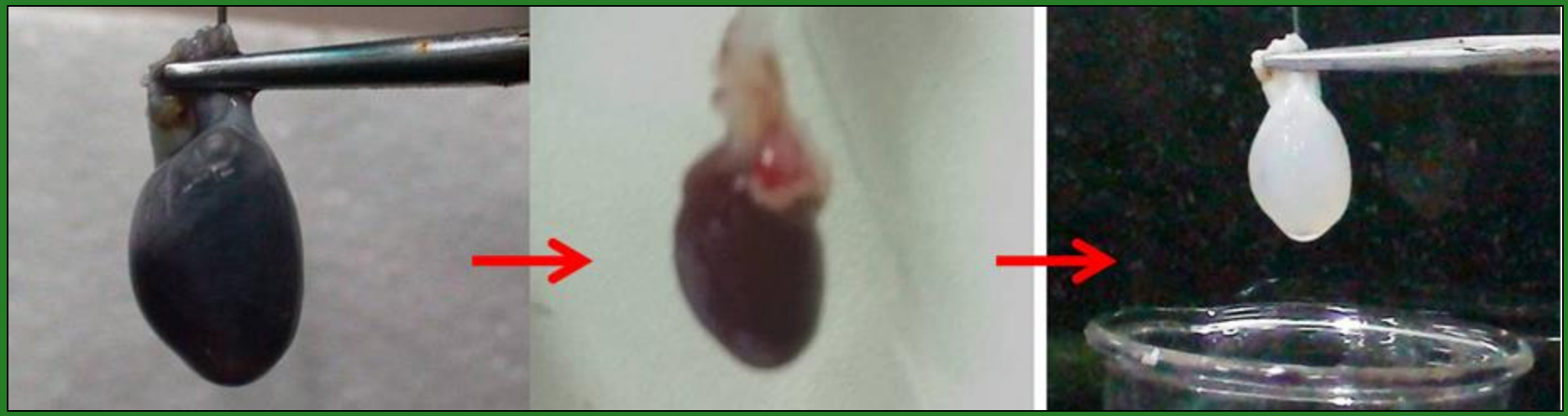
Type of functional cells

Bioactive molecules

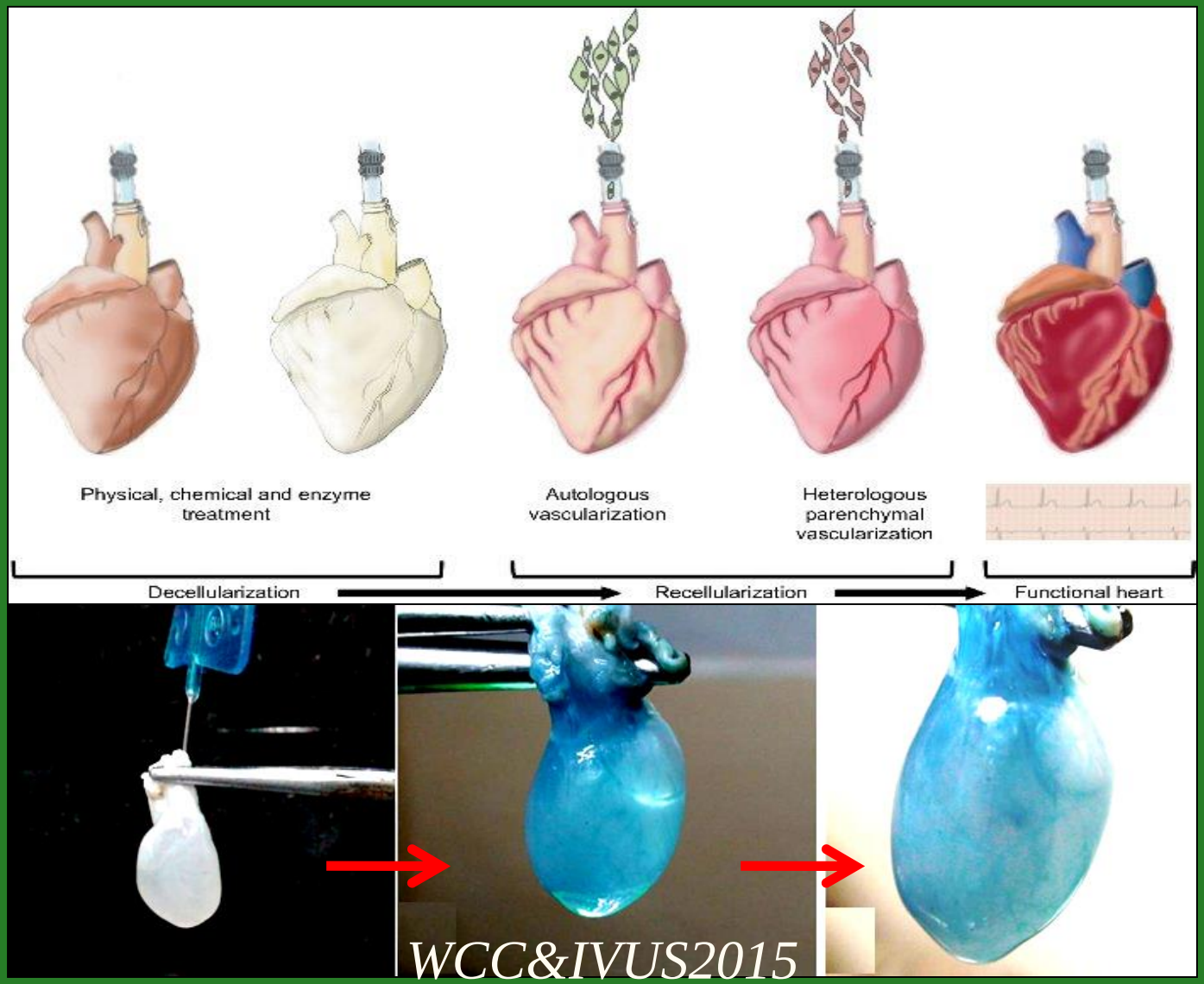
Decellularization & Stem cells Repopulation Technology

- Technology of removing cells and its components from an organ followed by repopulation of cells within the organ scaffold has been termed as Decellularization and Recellularization
- This technology provides
  - ❖ 3D-Exra cellular matrix (ECM)
  - ❖ Natural organ architecture and
  - ❖ Intact vascular tree

Development of xenogenic whole heart scaffold



Repopulation of whole decellularized Heart



Development of decellularized whole bioengineered organ scaffolds

Repopulation of decellularized whole organ scaffold using stem cells: an emerging technology for the development of neo-organ

Aleem Ahmed Khan, Sandeep Kumar Vishwakarma, Avinash Bardia & J. Venkateshwarulu

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Original Research

Preparation of natural three-dimensional goat kidney scaffold for the development of bioartificial organ

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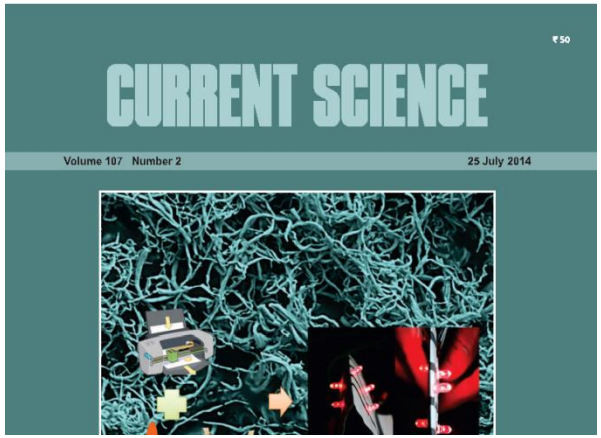

Decellularized heart: a step towards creating personalized bioengineered organs

Shortage of available organs for transplantation in end-stage organ failure has become a major challenge for organ transplantation. Annually, more than 1,000,000 patients die for the want of an organ. Further, in the case of patients who do receive organs, not all transplants are successful due to rejection and other complications. Even if a patient receives perfect match, it can still be rejected and he needs life-long use of immunosuppressors. With this background, for developing appropriate tissue engineering technologies, scientists are struggling to regenerate the whole organ.

Decellularized whole organ represents a new approach to provide three-dimensional architecture and complex natural heart extracellular matrix (ECM). The classical approaches for generating heart tissues have limited applications because of absence of three-dimensional architecture to support the rebuilding of muscles and vascular structures. Decellularization is an emerging technology to fulfil these promises. It is defined as the

tion of transplanted cells in the new-born organ. The vascular bed in the decellularized bioscaffold allows rapid delivery of oxygen and nutrients.

Discovery of stem cells has boosted confidence in creating bioartificial organs. The major limitations of stem cells are that they need the right architecture, environment and engraftment to perform the function. Ott *et al.*[1] published a landmark paper, which showed perfusion-decellularized bioengineered heart matrix by seeding cardiac and endothelial cells. To establish function, they maintained constructs in a bioreactor. By day-8 under physiological load and electrical stimulation, constructs could generate pump function in a modified working heart preparation. Following this, Ng *et al.*[2] implanted embryonic stem cells in decellularized rat heart. After 14 days, these developed into two different types of cells found in the heart: cardiac-marker expressing cells and endothelial or blood vessel cells. The cell-laden scaffold was then implanted back

human multipotential cardiovascular progenitor cells.

In summary, the concept of decellularized whole-heart bioengineering approach would revolutionize *in vitro* studies for early events of heart development, which after further advancements may find application in preclinical testing and development of personalized bioartificial heart.

1. Ott, H. C., Matthiesen, T. S., Goh, S. K., Black, L. D., Kren, S. M., Netoff, T. I. and Taylor, D. A., *Nature Med.*, 2008, 14(2), 213–221.
2. Ng, S. L. J., Narayanan, K., Gao, S. and Wan, A. C. A., *Biomaterials*, 2011, 6, 65.
3. Lu, T. Y., Lin, B., Kim, J., Sullivan, M., Tobita, K., Salama, G. and Yang, L., *Nature Commun.*, 2013, 4, 2307.

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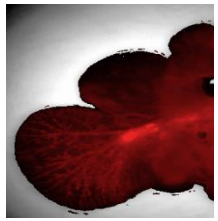

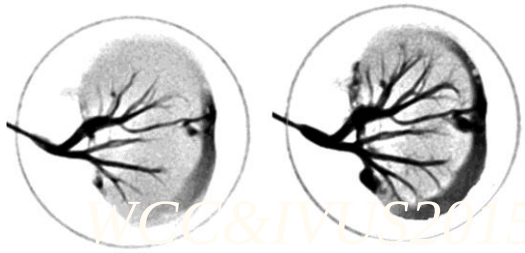

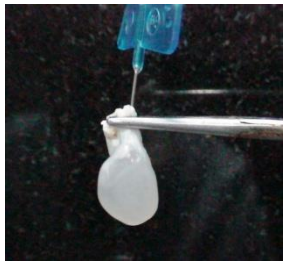

Conclusions

- UCB is an unlimited source of stem cells from biological waste
- Collection of cord stem cells is risk free to mother and baby
- Cord blood stem cells have a greater ability to differentiate into other cell types
- These cells have longer growth potential and have been shown to have a greater rate of engraftment
- Cord blood stem cells are much more tolerant to HLA tissue mismatching than bone marrow therefore leading to lower rate of GVHD

Future Directions

- Need to have dedicated specialized centers for preparation and delivery of cells under one roof
- Improvement in cell delivery techniques for better engraftment of transplanted cells
- Tools/ biomarkers for tracking of transplanted cells
- Repopulating decellularized xenogenic heart could be a potential technology of neo-organ development

Our Research Team

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Thank You
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