BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biomedical science

Course Title : Stem Cell Biology & Tissue Engineering

Course Code : 18BMS48C14

Unit-II

TOPIC: STEM CELL AND REGENERATIVE MEDICINE

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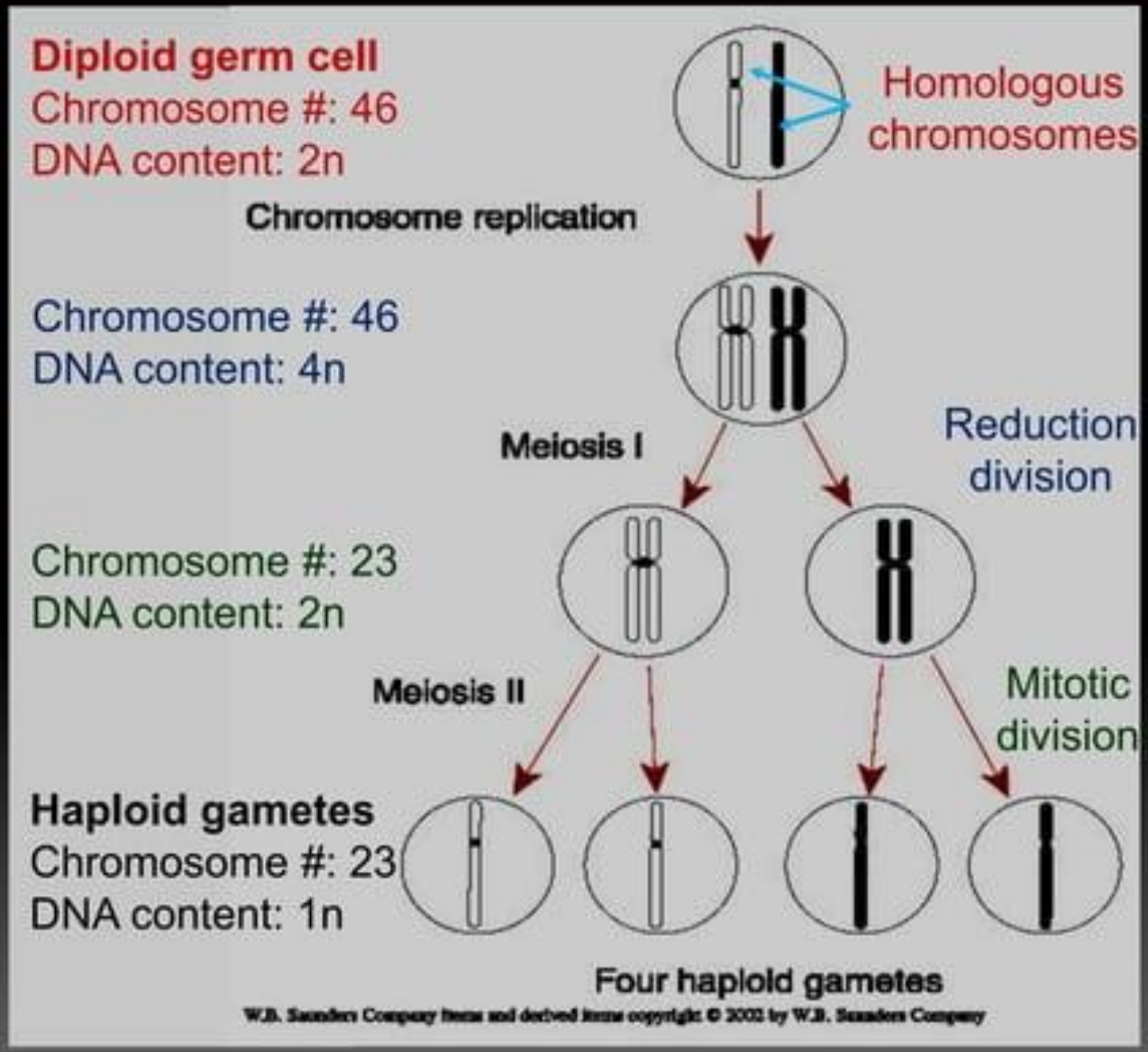
Guest lecturer
Department of Biomedical Science

STEM CELL AND REGENERATIVE MEDICINE

What happens after meiosis?



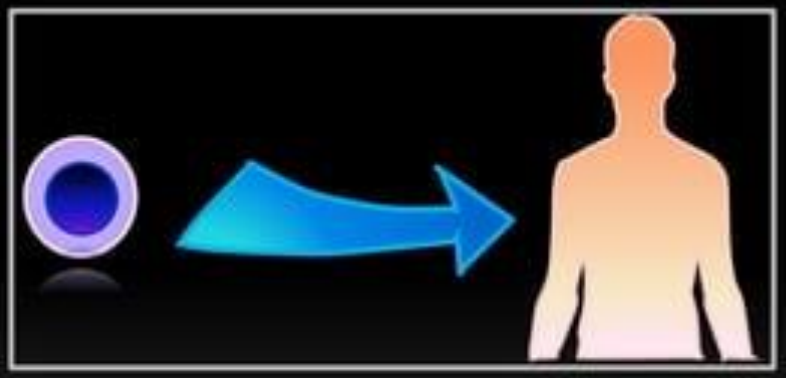
How does a one cell zygote become a human?

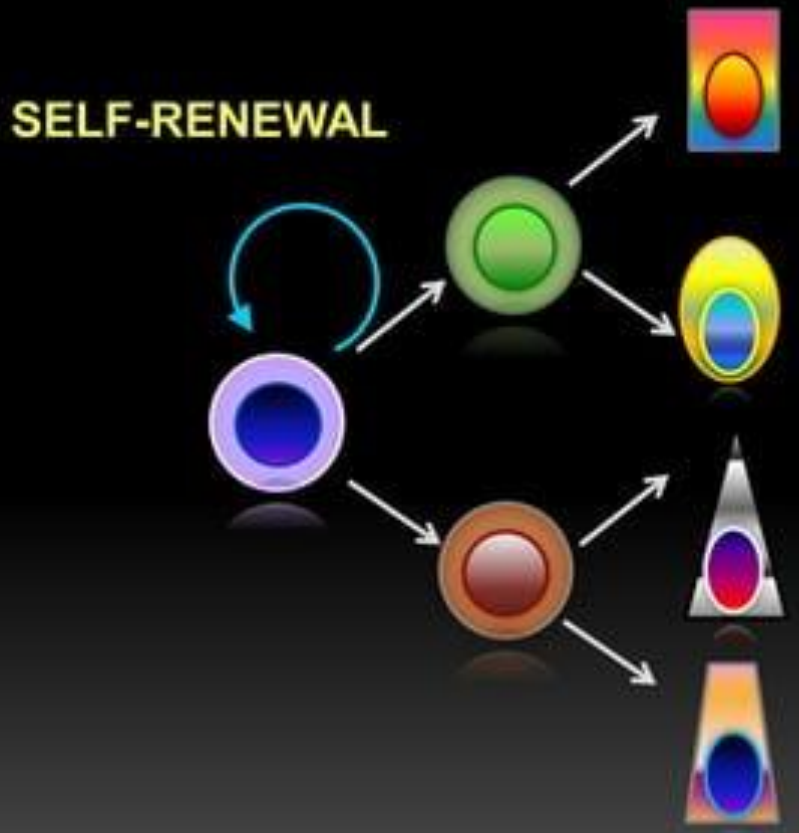
The fusion of a haploid male gamete and female gamete leads to the formation of a zygote. A zygote is a fertilized egg.

Embryogenesis

http://embryo.soad.umich.edu/carnStages/carnStages.html
Image courtesy of Brad Smith, University of Michigan

Role of Stem Cells





The history of a medical sensation

1981: Martin Evans at the University of Cambridge is first to identify embryonic stem cells – in mice

Nobel Prize 2007

1997: Ian Wilmut and colleagues at the Roslin Institute, Edinburgh. Dolly the sheep, the first artificial animal clone.

1998: James Thomson (University of Wisconsin) and John Gearhart (Johns Hopkins) isolated human embryonic stem cells and grew them in the lab.

2001: Bush controversy

2006: Shinya Yamanaka of Kyoto University reprograms ordinary adult cells to form "induced pluripotent stem cells".

Nobel Prize 2012

2009: Obama-power
2010: Medical treatment of Spinal injury using hESCs
2012: Medical treatment of Blindness using hESCs
2014: Human trials using iPSCs

Ref: http://www.newscientist.com/article/dn24970

Some important stem cell jargon

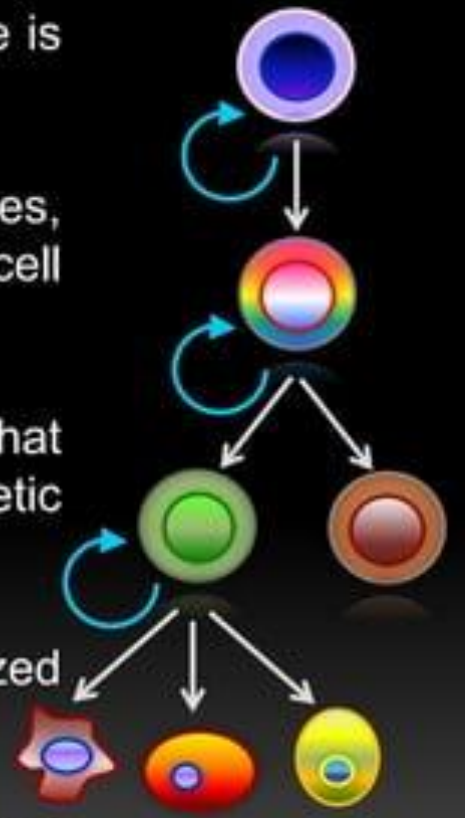
Self renewal: The ability of a stem cell to divide and produce copies of itself for an indefinite period of time.

Totipotent: A cell capable to form entire organism. The zygote is totipotent; not demonstrated for any vertebrate stem cell.

Pluripotent: A cell able to form all the body's cell lineages, including germ cells, and some or even all extraembryonic cell types. Example: embryonic stem cells.

Multipotent: A cell able form multiple mature cell types that constitute an entire tissue or tissues. Example: haematopoietic (blood) stem cells.

Differentiation: The process by which cells become specialized to perform particular tasks.



Regenerative medicine: Reconstruction of diseased or injured tissue by activation of resident cells or by cell transplantation.

Ref: http://www.eurostemcell.org/stem-cell-glossary

Kinds of Stem Cells

Embryonic stem cells come from a five to six-day-old embryo. They have the ability to form virtually any type of cell found in the human body.

Adult stem cells are undifferentiated cells found among specialized or differentiated cells in a tissue or organ after birth.

Comparison of Embryonic v/s Adult Stem Cells

Embryonic Stem Cells

- Cell lines last and last and last
- Multipotent
- Easy to find
- Ethical issues - when does life begin?

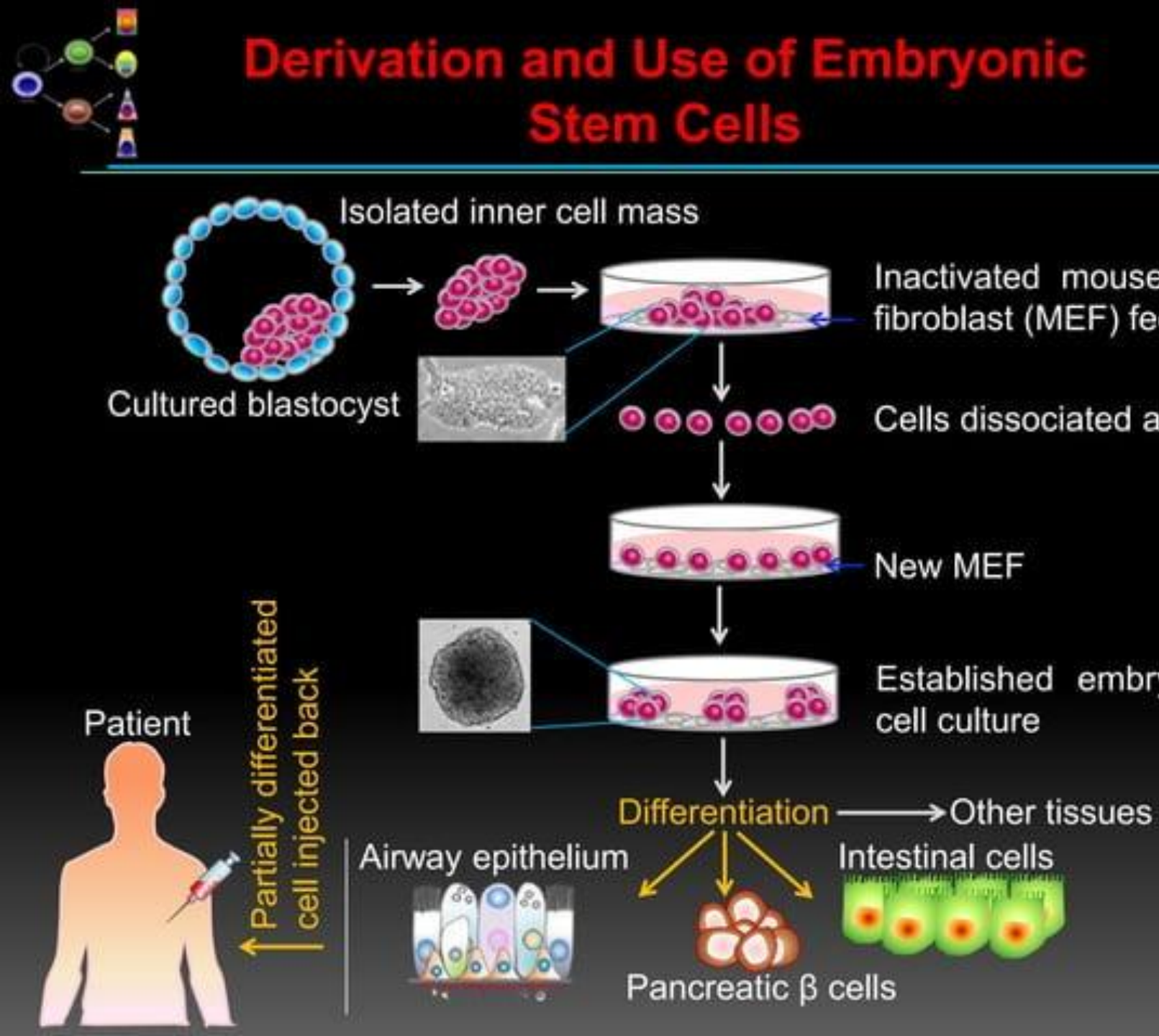
Adult Stem Cells

- Cell lines do not last
- Not multipotent
- Hard to locate
- No ethical issues

Embryonic Stem Cell

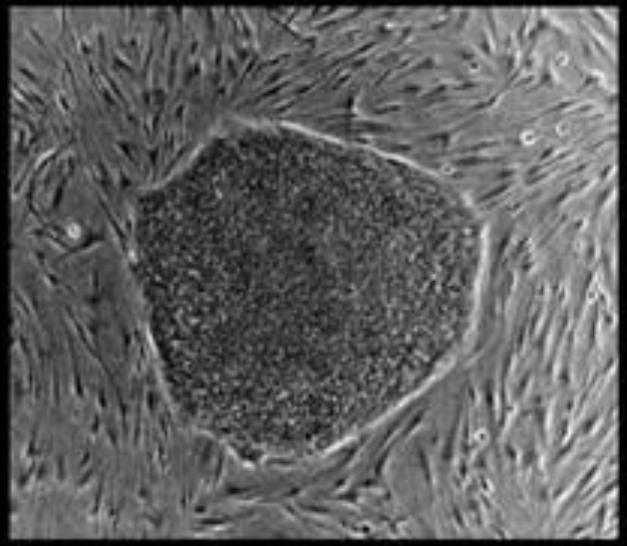
- Donated excess *In vitro* fertilization (IVF) embryos.
- Can be grown indefinitely in the laboratory in an unspecialised state retain ability to specialise into many different tissue types – know as pluripotent.
- Can restore function in animal models following transplantation.

Derivation and Use of Embryonic Stem Cells



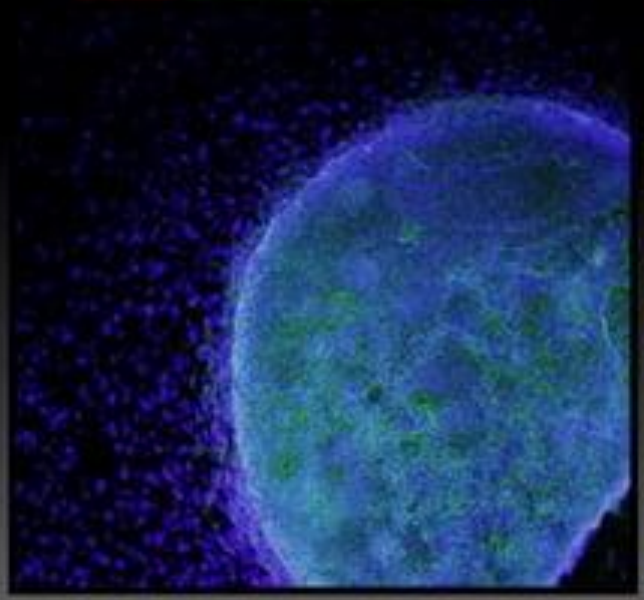
Derivation and Use of Embryonic Stem Cell Lines

What do cultured ES cells look like?

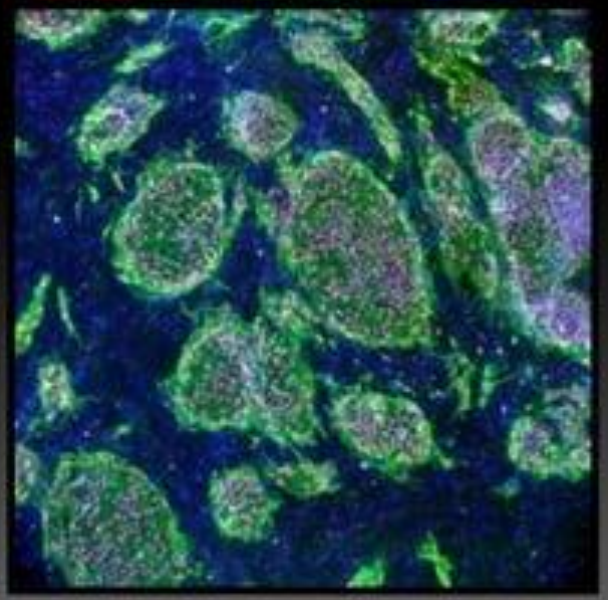


Fluorescent imaging of embryonic stem cell colonies.

Sox2 E-CADHERIN DAPI



Sox2 E-CADHERIN DAPI



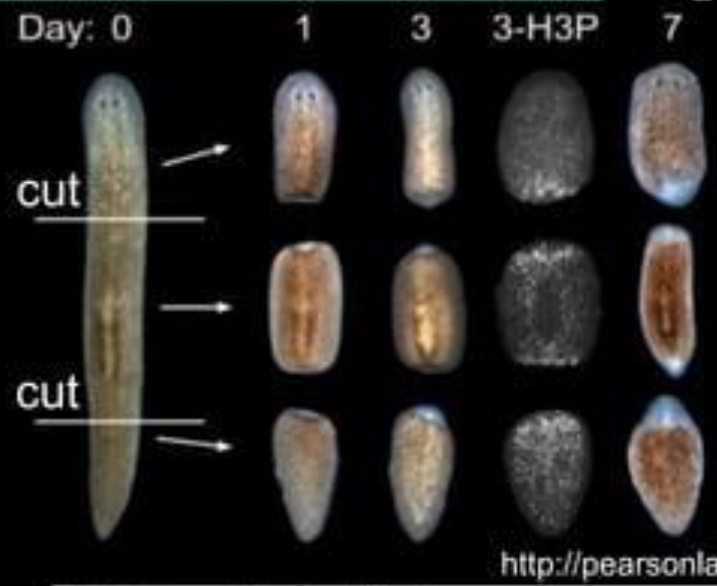
How to use stem cells ?
Will discuss later

What to do with stem cells?

Isolation	Characterization	Expansion	Differentiation
➢ Isolation of individual stem cell population using appropriate markers	➢ Characterization of isolated stem cell populations using additional markers	➢ Culturing stem cell lines in a stable, multi or pluripotent state, free from mutations and to sufficient quantity	➢ Activation of stem cell differentiation to desired lineages maintaining strict control
➢ Checks to ensure that stem cells retain their functionality and potential to differentiate	➢ Ensuring that cells are transplant ready	➢ Economical expansion to be aimed to make cell-therapy a reality	➢ Checking for functionally active differentiated cells

Adult stem cell
Regeneration …that we all know

- Outstanding Examples
  - Planarian
  - Newt
  - Embryos
- Inverse Relationship
  - Increase complexity
  - Decrease regenerative ability

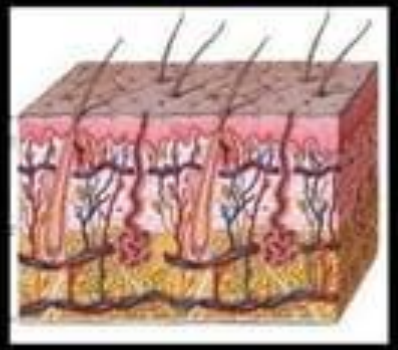


http://pearsonlab.ca

http://www.medgadget.com

Regeneration in Humans

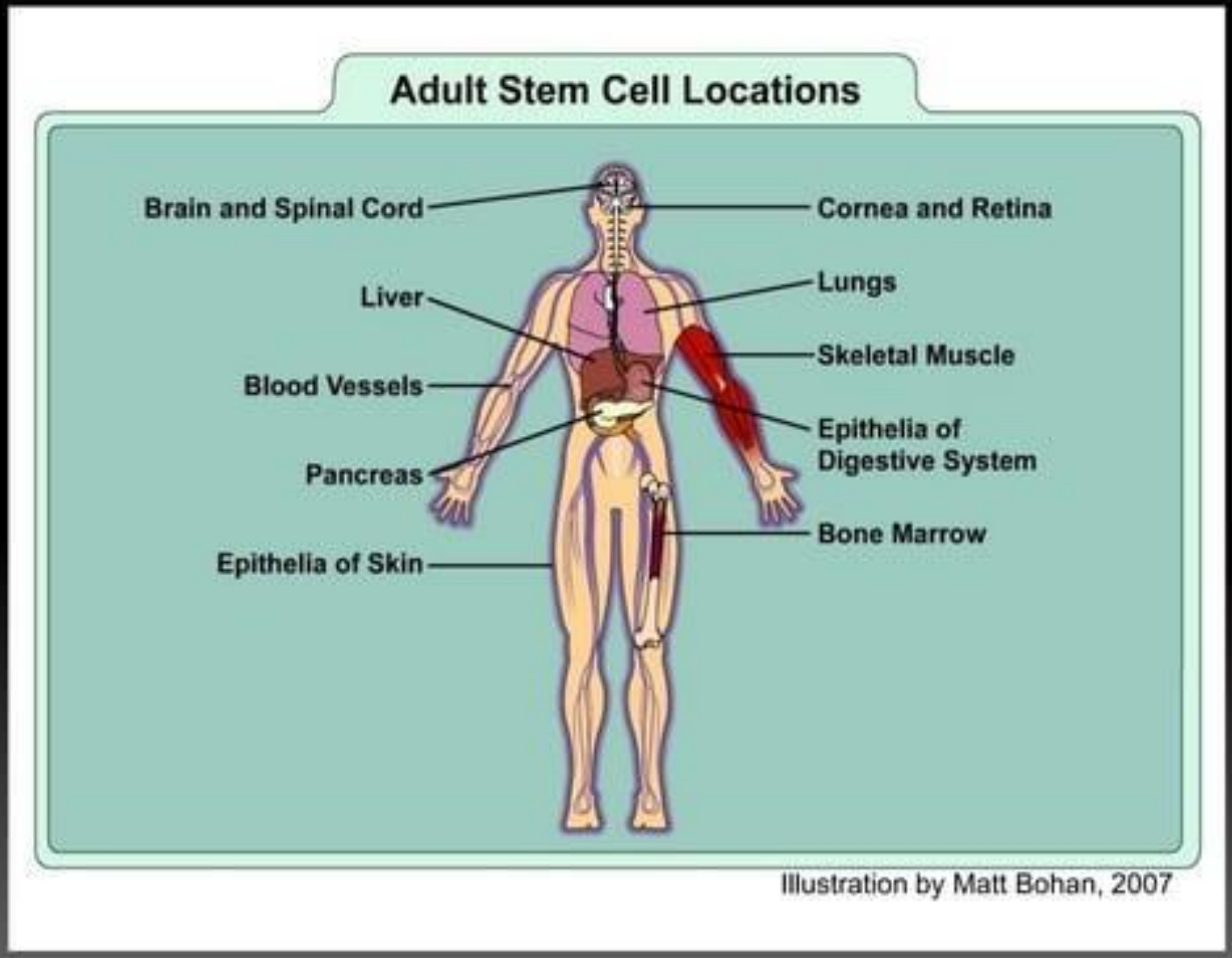
High



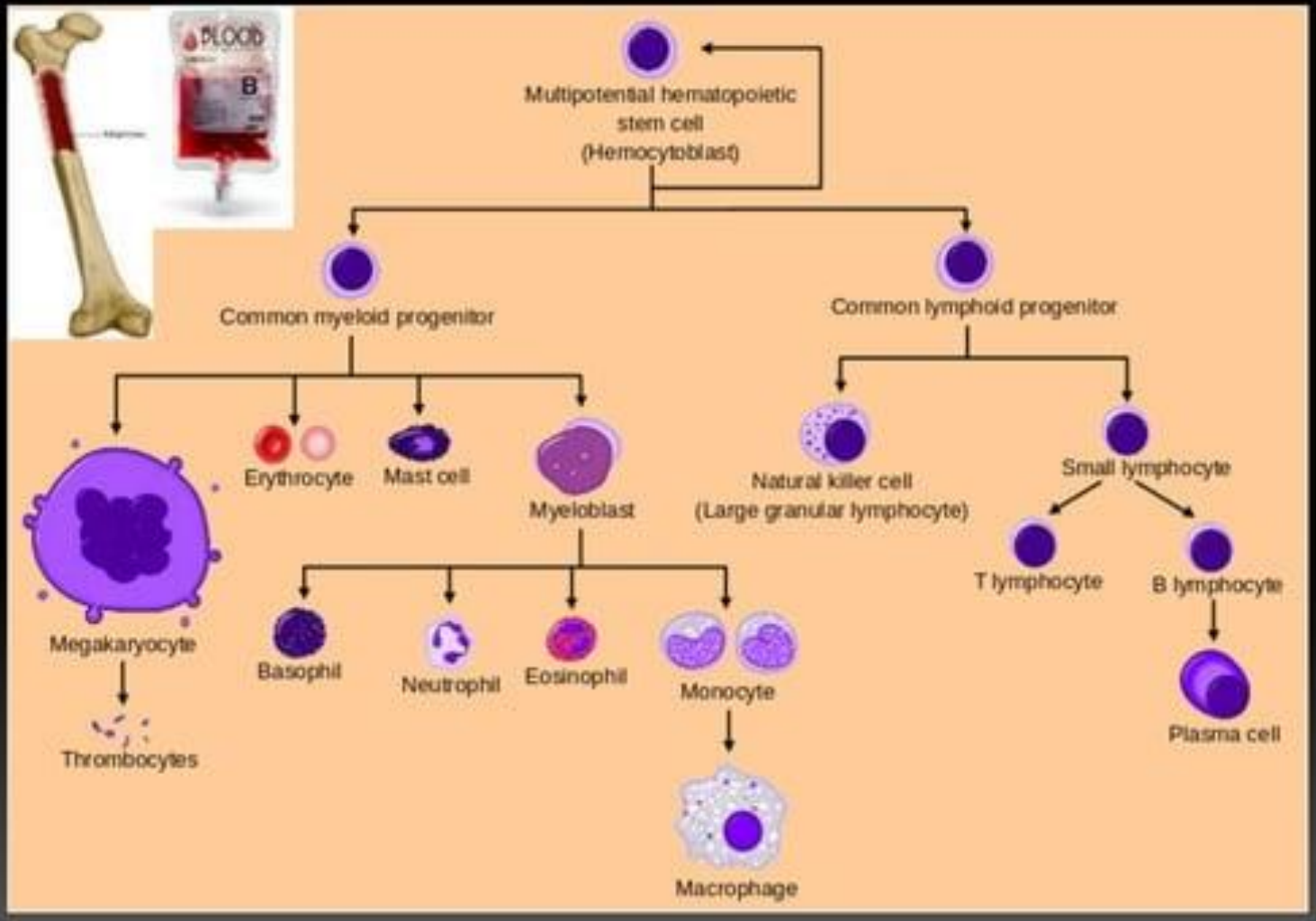
Moderate

Low

Adult Stem cells have also been discovered in multiple organs

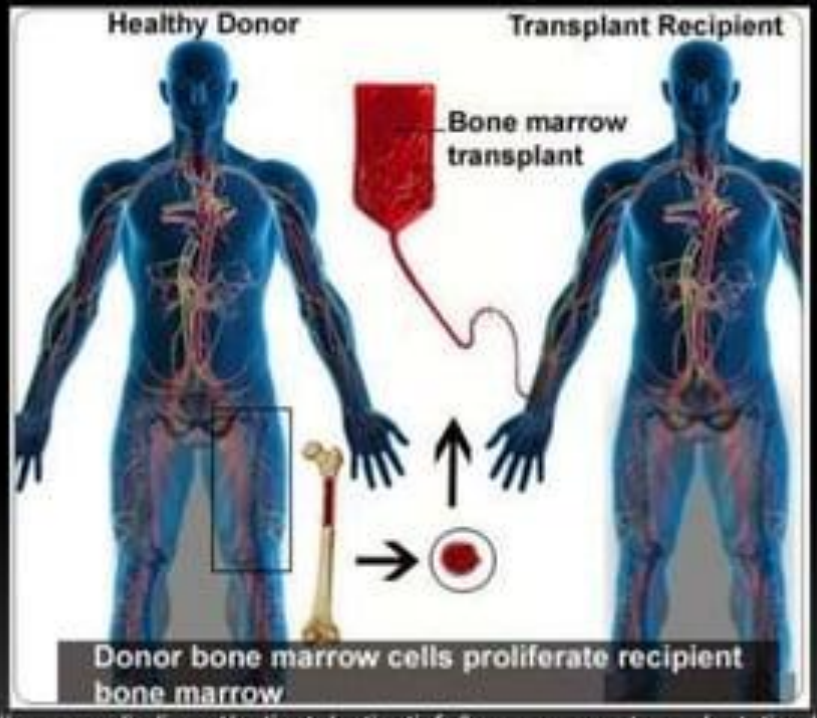


Hematopoietic Stem Cell (HSC)



Hematopoietic Stem Cell use

- Bone Marrow Transplants (BMT) & hematopoietic stem cell therapy (HCT)
  - Lymphomas and thymomas
  - Hematopoietic cells
  - Metastatic cancers of other origins
- Autoimmune Diseases with hematopoietic stem cells (HSCs)
  - Rheumatoid arthritis
  - Systemic Lupus Erythematosus
  - Type 1 diabetes mellitus
  - Multiple sclerosis
  - Pernicious anemia



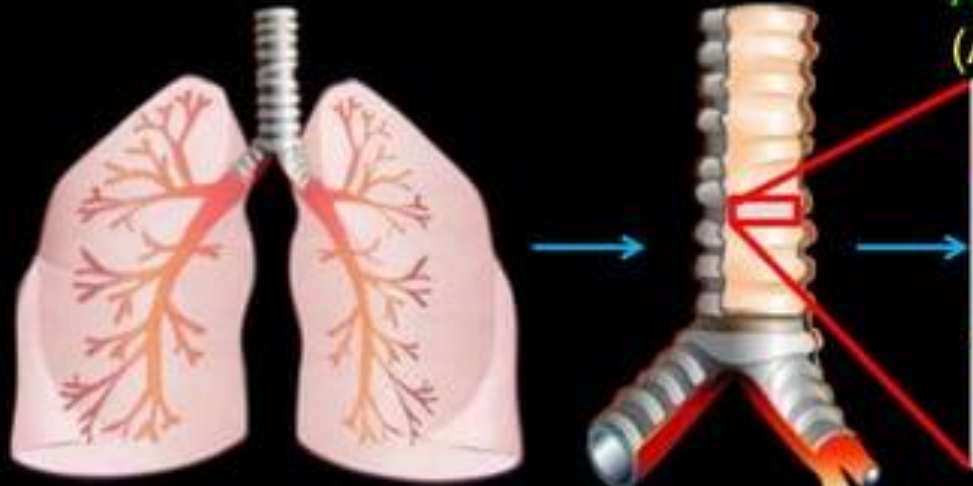
http://www.medindia.net/patients/patientinfo/bone-marrow-transplantation.htm

- Hematopoietic Stem Cell: Best-studied, used clinically for 30+ years
- Allogeneic and Autologous stem cell transplantation

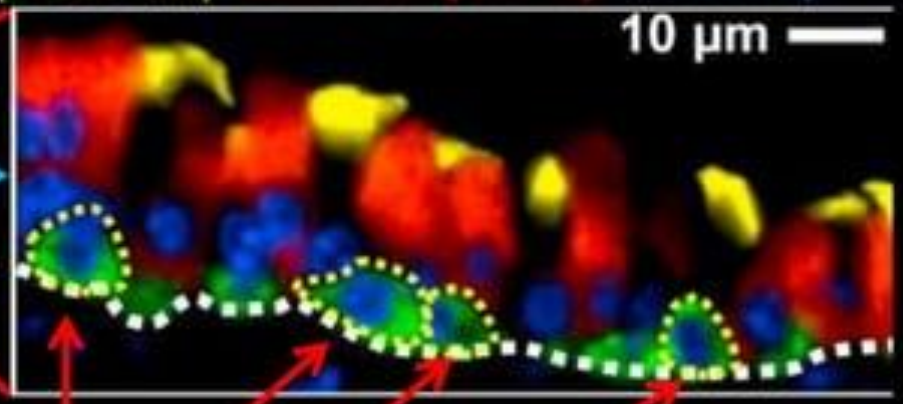
Umbilical cord stem cells (UCS cells): an alternate source of stem cells

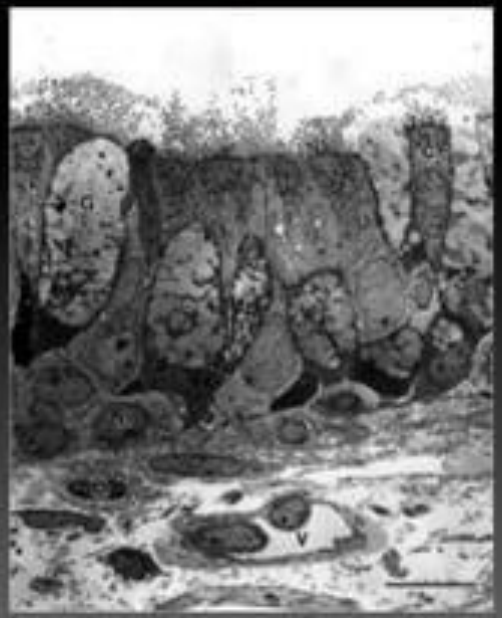
- Also Known as Wharton's Jelly
- Adult stem cells of infant origin
- Isolated prior to/ immediately following birth
- Haematopoietic stem cells (Majority)
- 100,000 stem cells per mL in UCB
- Alternate to bone marrow stem cells

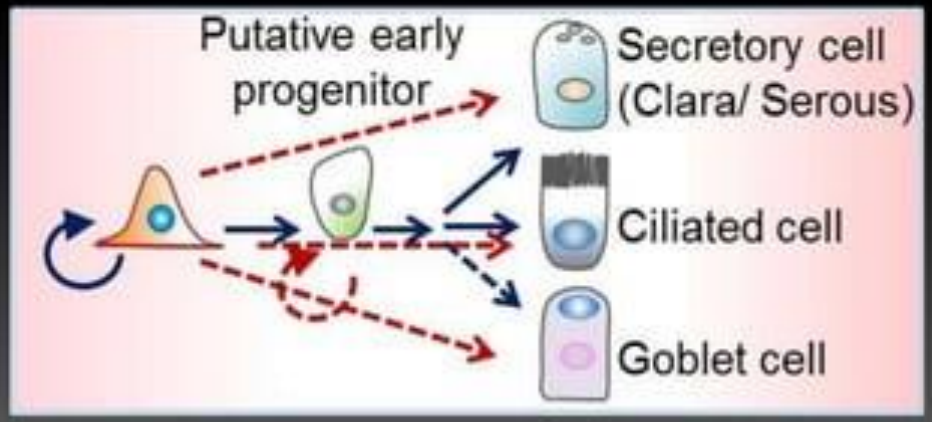
Adult Stem cell in airway



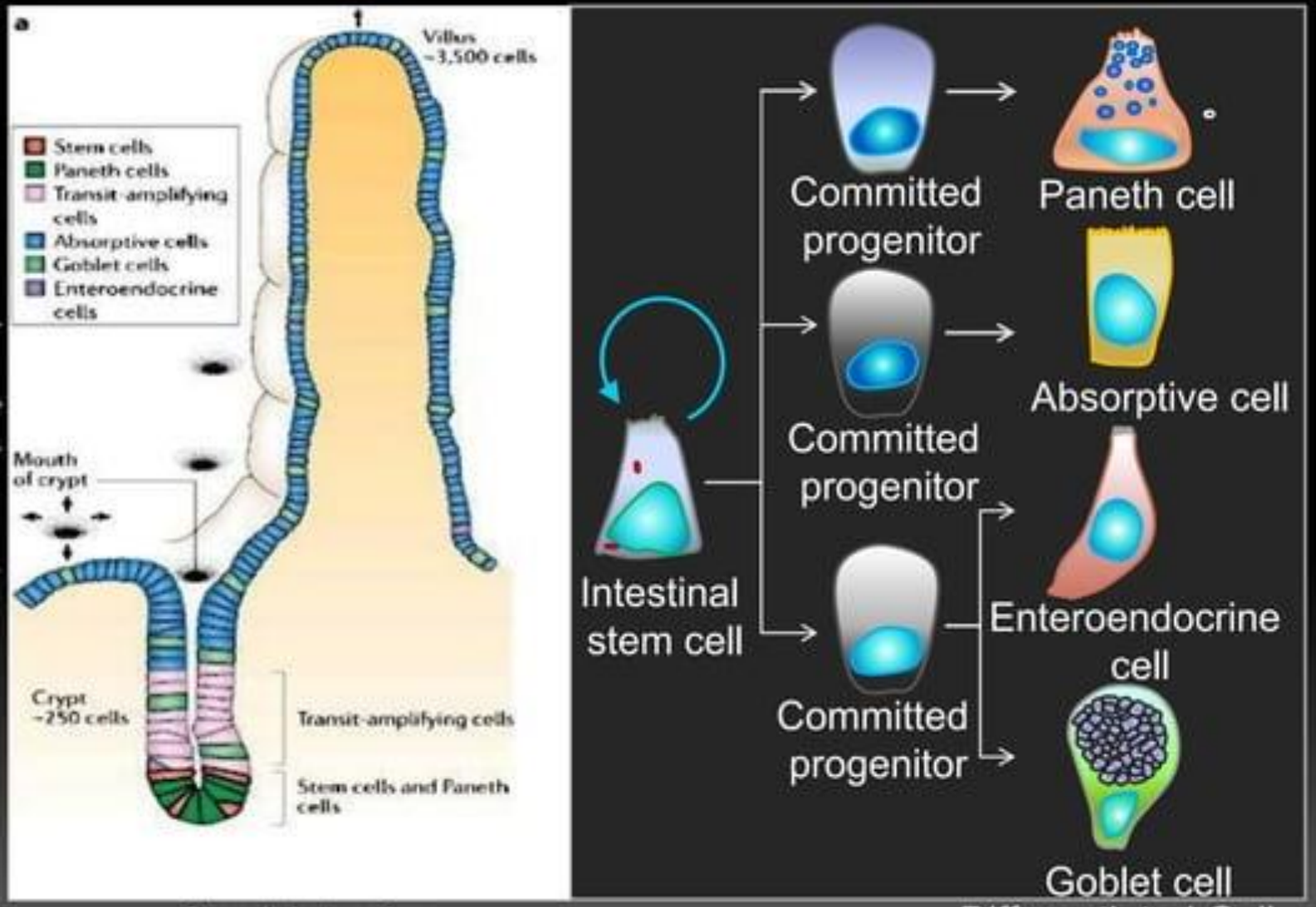
Airway Basal Stem Cell (Krt5) Ciliated cell (Acet-Tub) Clara cell (CC10) Nucleus (DAPI)







Intestinal stem cells and therapy



Nature Reviews Genetics 7, 349-359 (May 2006)

What are stem cell technologies?

- Cloning technologies
  - Is human cloning a technology?
  - What is different about cloning embryonic stem cells?

- Induced Pluripotent Stem cells
  - New ways to potentially avoid the use of embryos
  - Disease-specific stem cell lines created
  - The promise and potential pitfalls of this approach

When does research actually become technology?

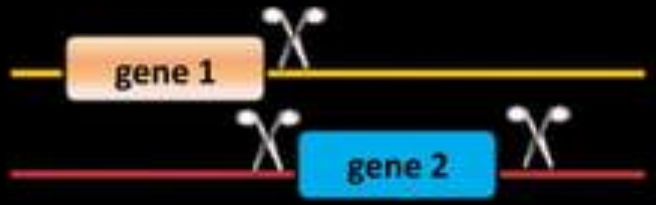
Cloning

There are two very different types of cloning:

Reproductive cloning

- Use to make two identical individuals
- Very difficult to do
- Illegal to do on humans

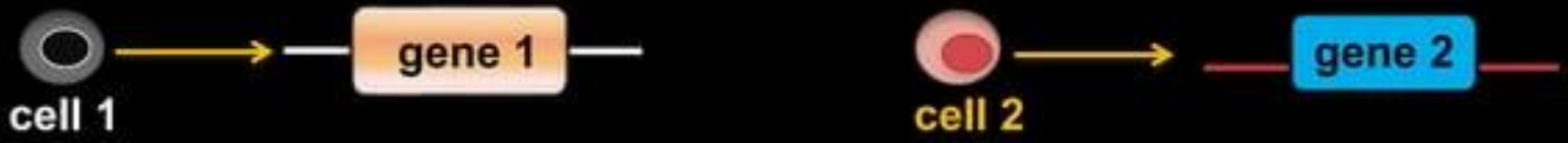
Molecular cloning



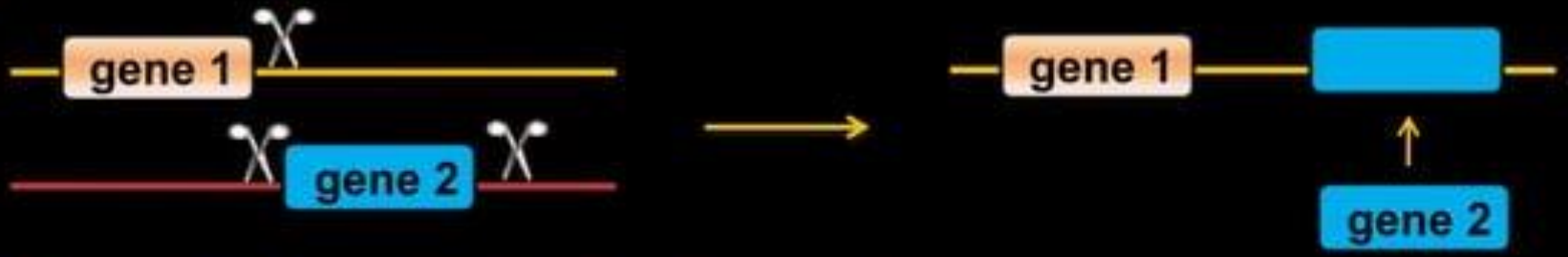
- Use to study what a gene does
- Routine in the biology labs
- Cut and paste

Molecular cloning: Principles

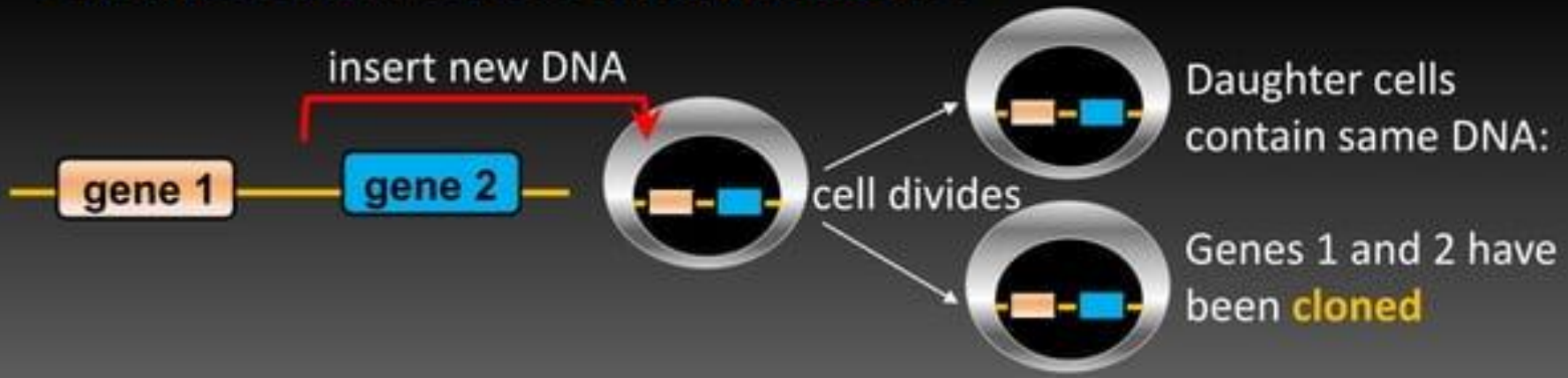
1) Take DNA out of the nucleus



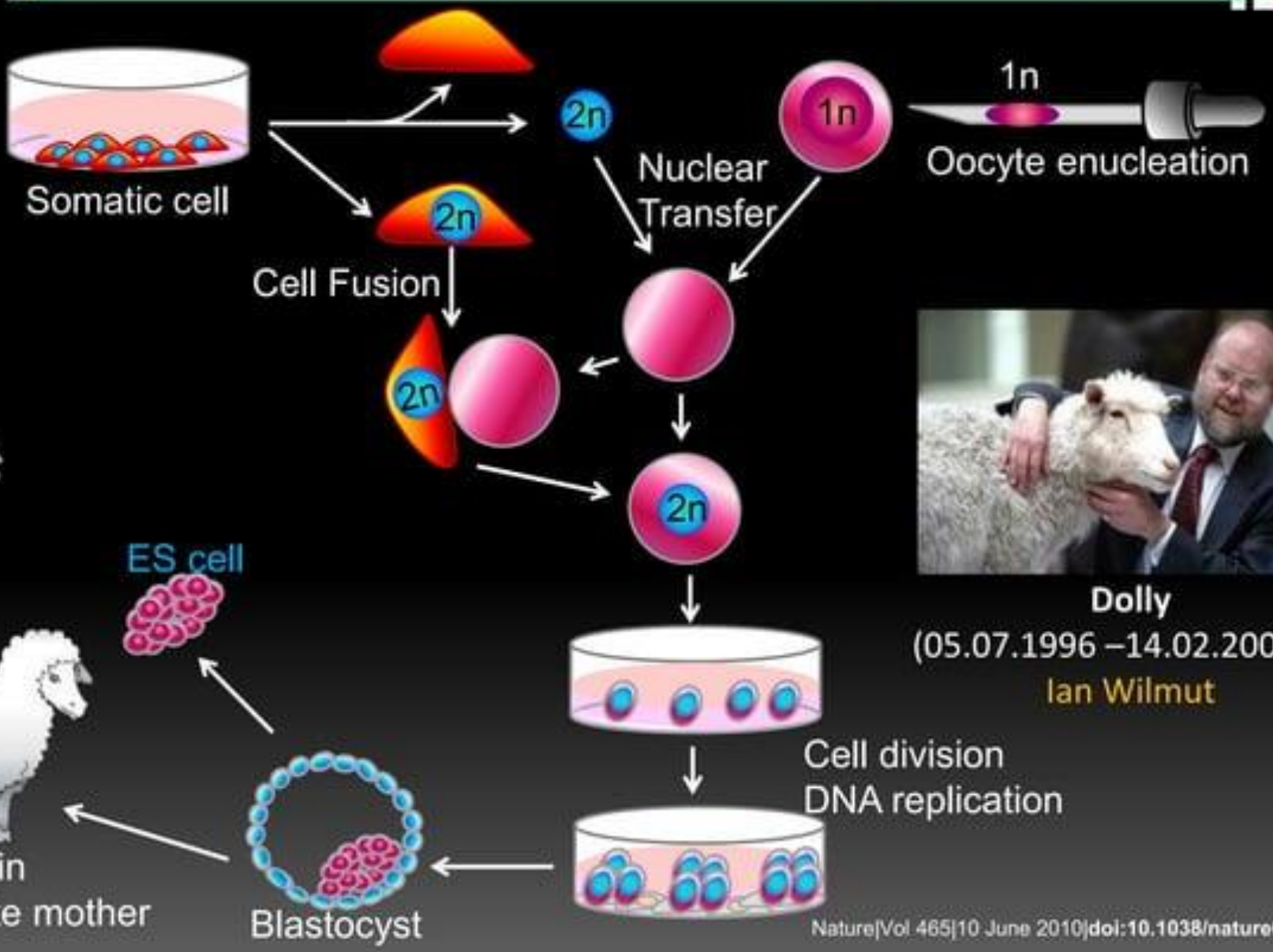
2) Make a new piece of DNA



3) Put new DNA into a test cell and grow copies



Somatic Cell Nuclear Transfer (SCNT)



Nature|Vol 465|10 June 2010|doi:10.1038/nature09229.

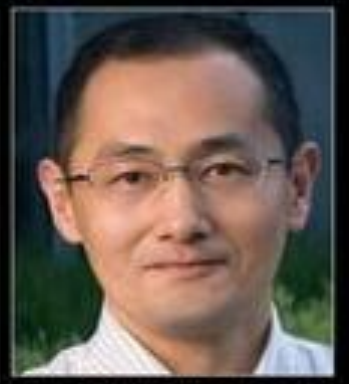
Induced Pluripotent Stem Cells (iPSCs)

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

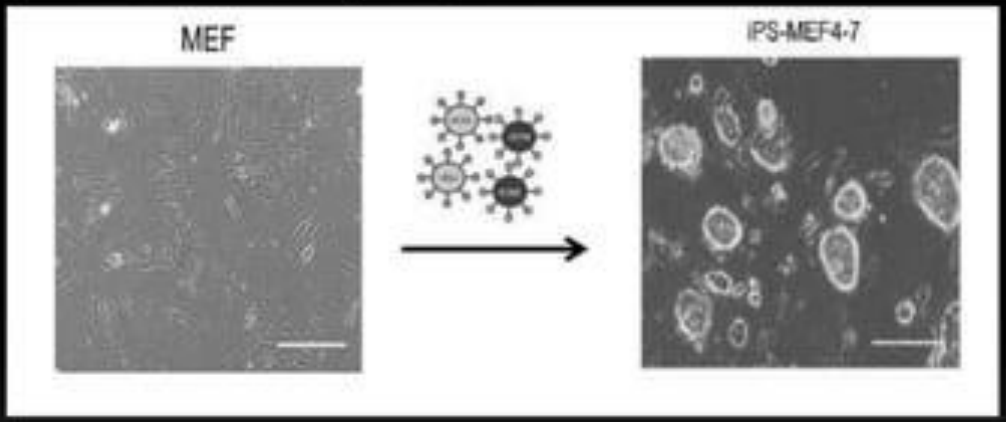
Cell

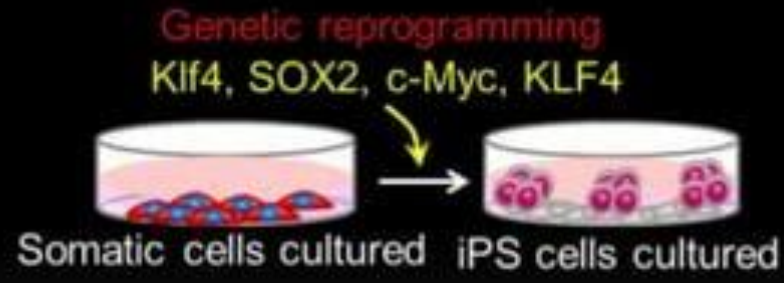
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]

Cell 126, 663–676, August 25, 2006



Dr. Shinya Yamanaka





Dr. Kazutoshi Takahashi

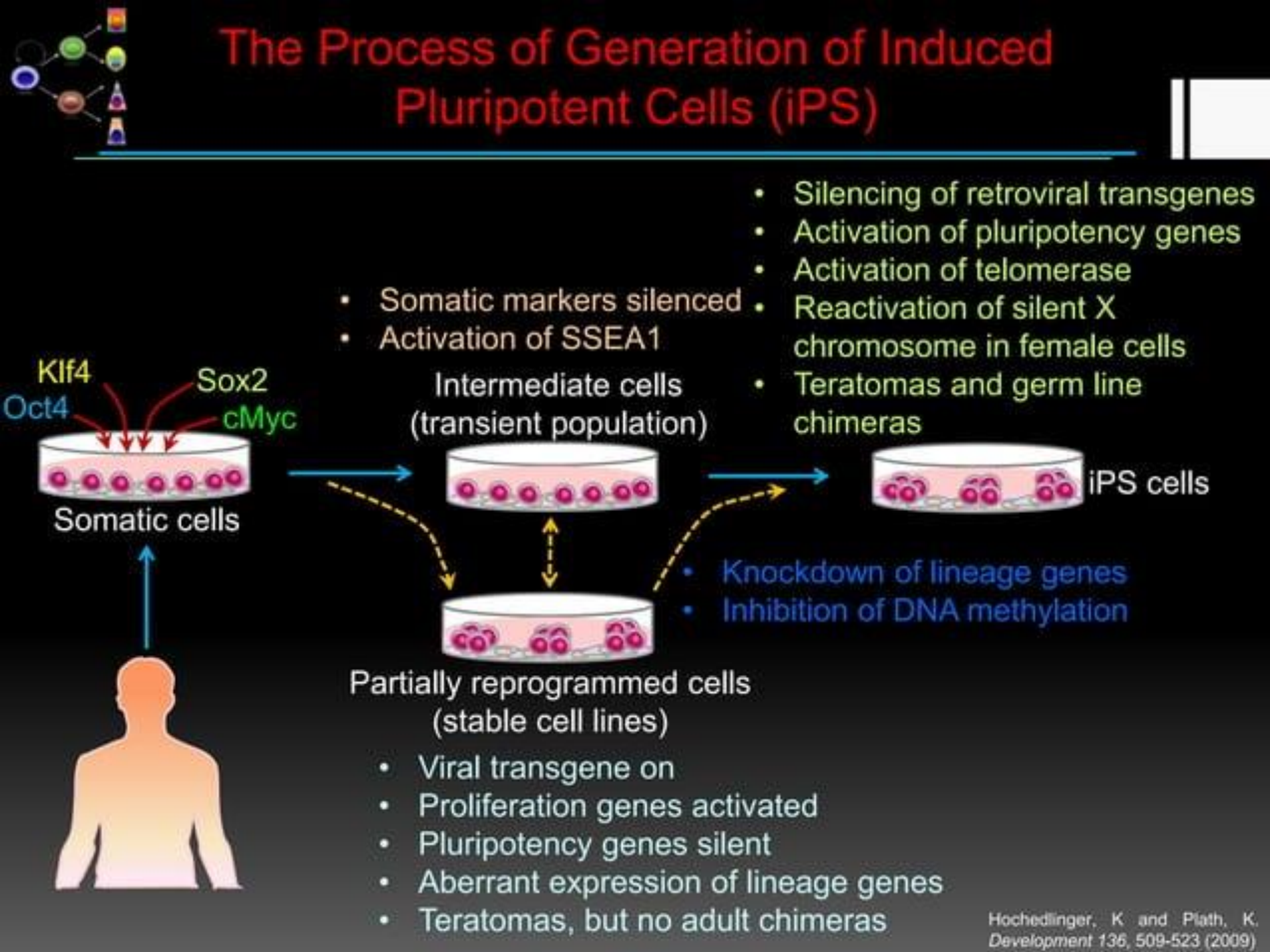
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Cell

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

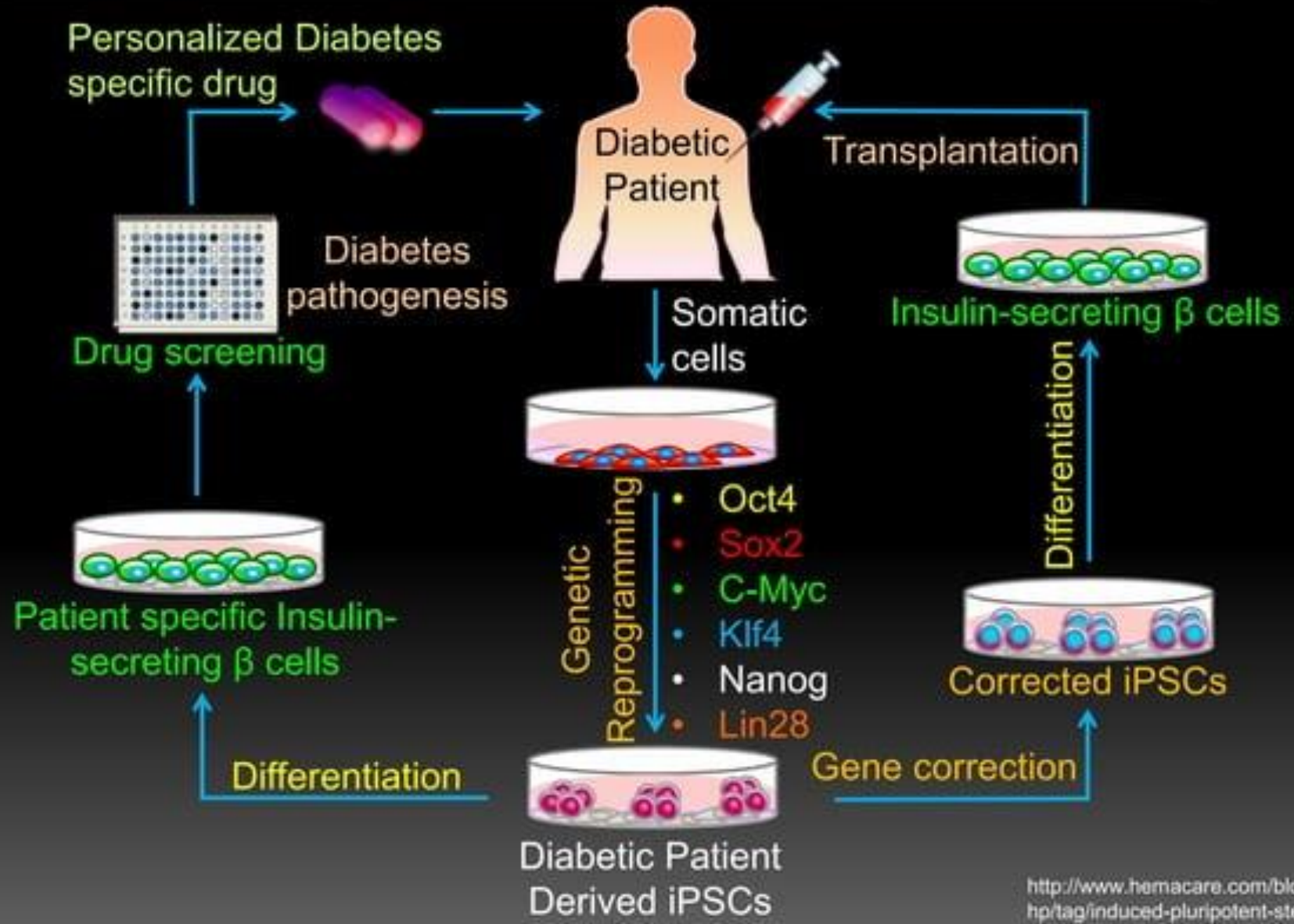
Cell 131, 861–872, November 30, 2007

The Process of Generation of Induced Pluripotent Cells (iPS)



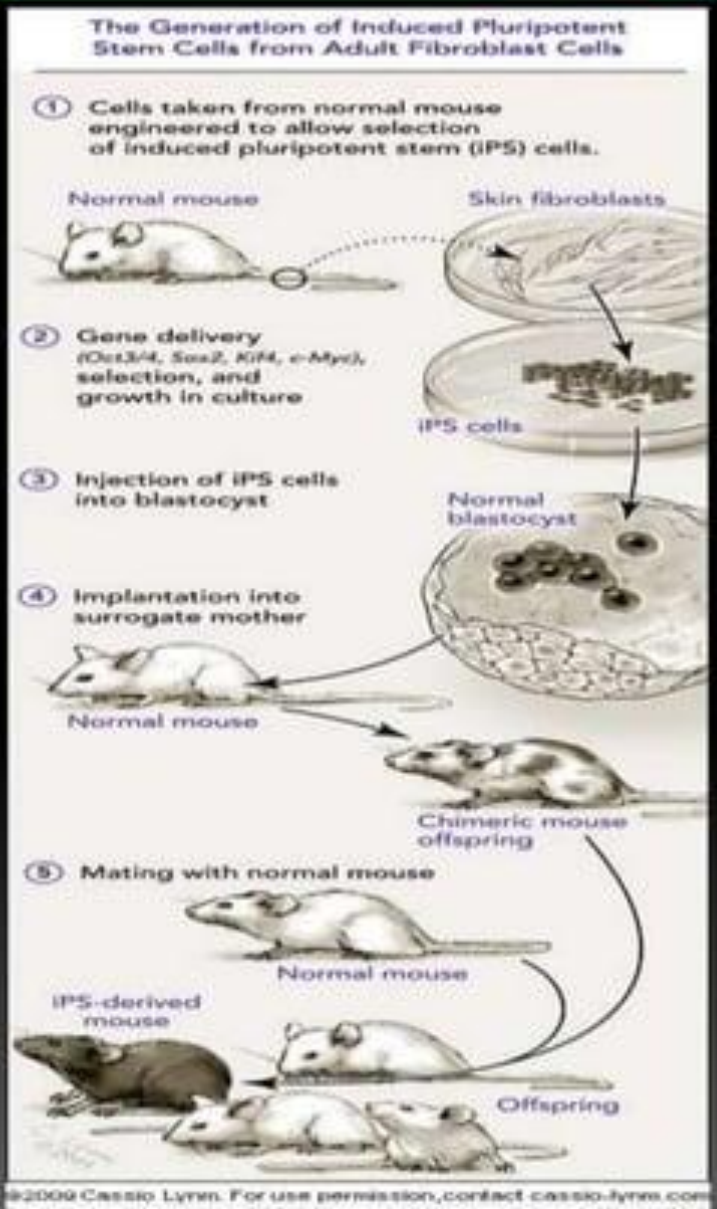
Hochedlinger, K and Plath, K. *Development 136*, 509-523 (2009)

How to use Induced Pluripotent Cells (iPS)



http://www.hemacare.com/blog/index.php/tag/induced-pluripotent-stem-cells/

Evaluation of iPSCs by the chimera formation.

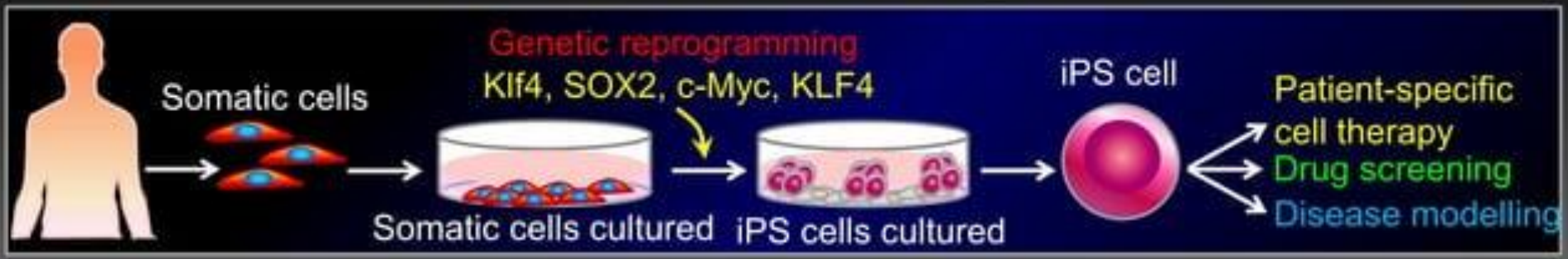


iPSCs has been generated from

Mouse (*Yamanaka et al.*, 2006)
Humans (*Yamanaka et al.*, 2007)
Rhesus monkey (*Liu et al.*, 2008)
Rats (*Liao et al.*, 2009; *Li et al.*, 2009)
Canine (*Shimada, H. et al*, 2010)
Porcine (*Esteban, M. A. et al.*, 2009)
Marmoset (*Wu, Y. et al.*, 2010)
Rabbit (*Honda, A. et al.*, 2010)
Equine (*Kristina Nagy et al.*, 2011)
Avian (*Lu et al.*, 2011)

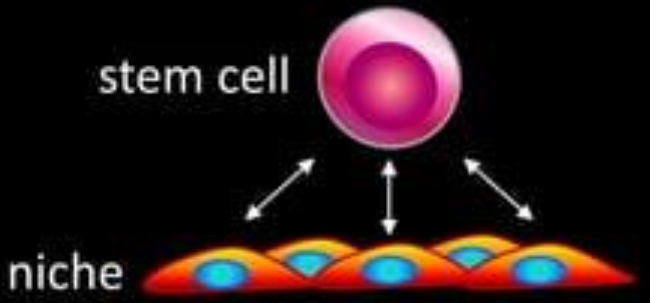
Advantages of Induced Pluripotent Cells (iPS)

- No need for embryos/ No ethical issues
- Ability to differentiate into many cell types
- Vastly renewable. Cell number not a limitation. iPSC cell lines can be made and stored
- Easily accessible. Variety of somatic cells such as blood, keratinocytes, fibroblast and hepatocytes etc can be used
- Can be used to model human disease in a dish and drug screening
- Individual-specific i.e. personalized or non-immunogenic



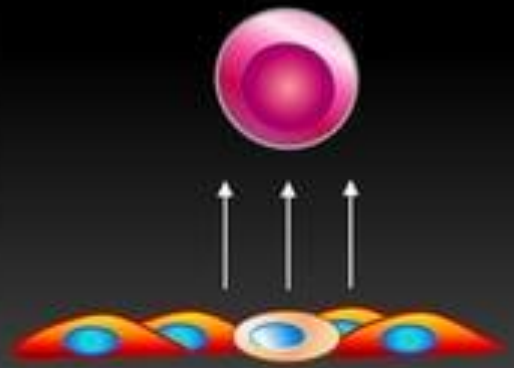
Stem cell home: Niche

Niche
Microenvironment around stem cells that provides support and signals regulating self-renewal and differentiation

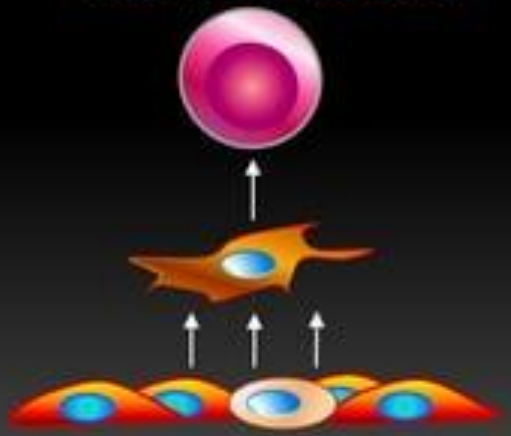


Direct contact

Soluble factors



Intermediate cell



Stem cell therapy: Challenges

embryonic stem cells/
iPSC/Adult stem cell

How to control stem cells after injection

Marker identification for isolating pure SCs pool

Finding right expansion conditions

Different Differentiation conditions

Different Scaffolding required

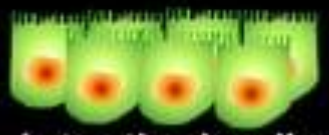
Different delivery system and monitoring

Long term storage for future use

Skin

Neuron

Blood



Intestinal cells

Airway epithelium

Pancreatic β cells

How Stem Cells Are Changing the Way We Think About Disease: Latest stories

http://www.dailymail.co.uk/sciencetech/article-2599568/UK-scientists-make-body-parts-lab.html

- Professor Alexander Seifalian (pictured) made a nose for a man who lost his to cancer.
- Stem cells were taken from the patient's fat and grown in the lab for 2 weeks before being used to cover the scaffold.
- Dr Michelle Griffin (pictured) said ears are harder to make than noses

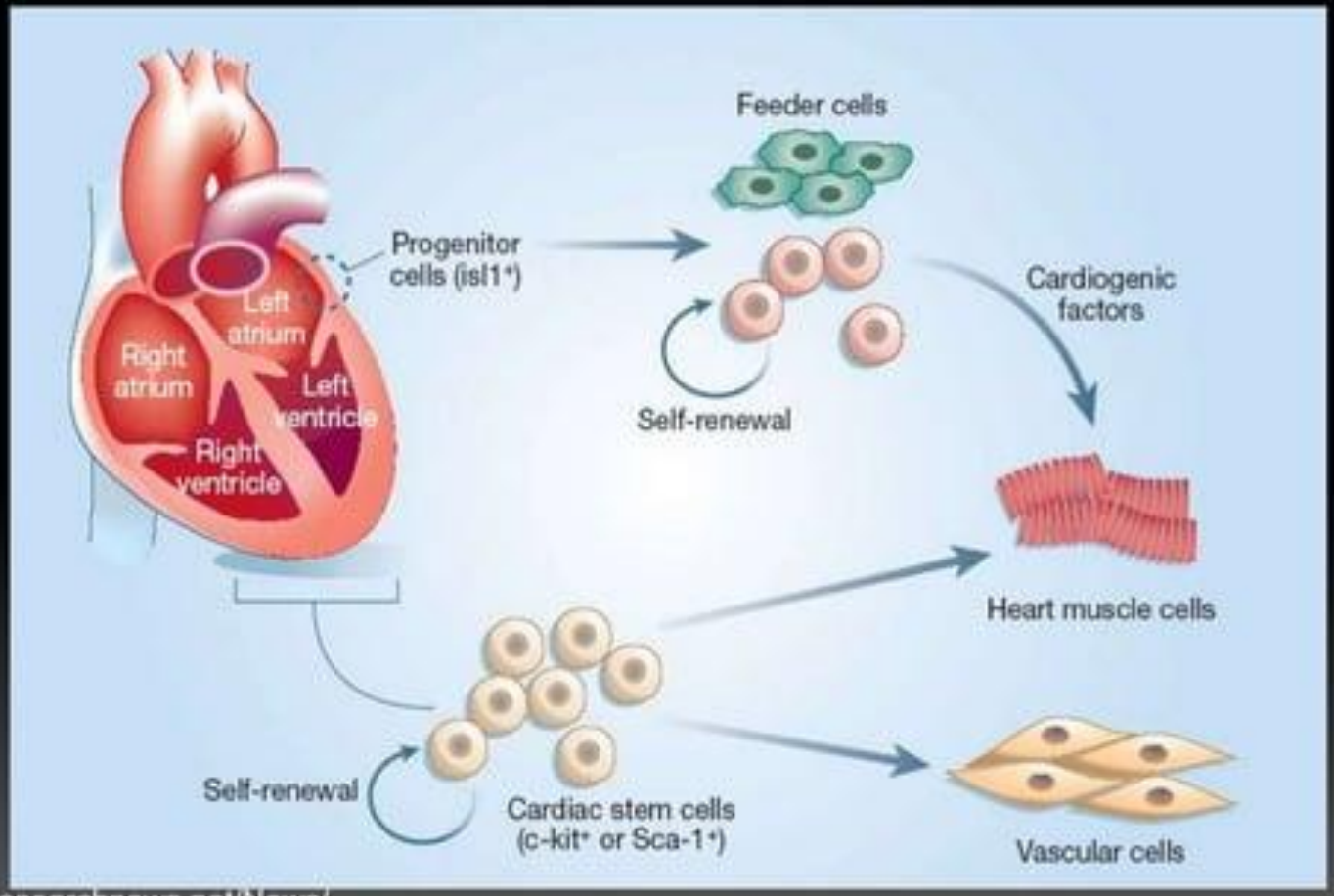
Stem cell based therapy

Paolo macchiarini (scaffolding, seeding) and Harvard bioscience

- Born without a windpipe
- Artifitial trachea grown from her own stem cells on a 3-inch-long frame of plastic fibers
- Bone marrow stem cells seeded on scaffold and grown in a bioreactor before transplantation
- Hannah died after three months of the surgery
- Though controversies exist but this story suggest the future use of stem cells for therapy

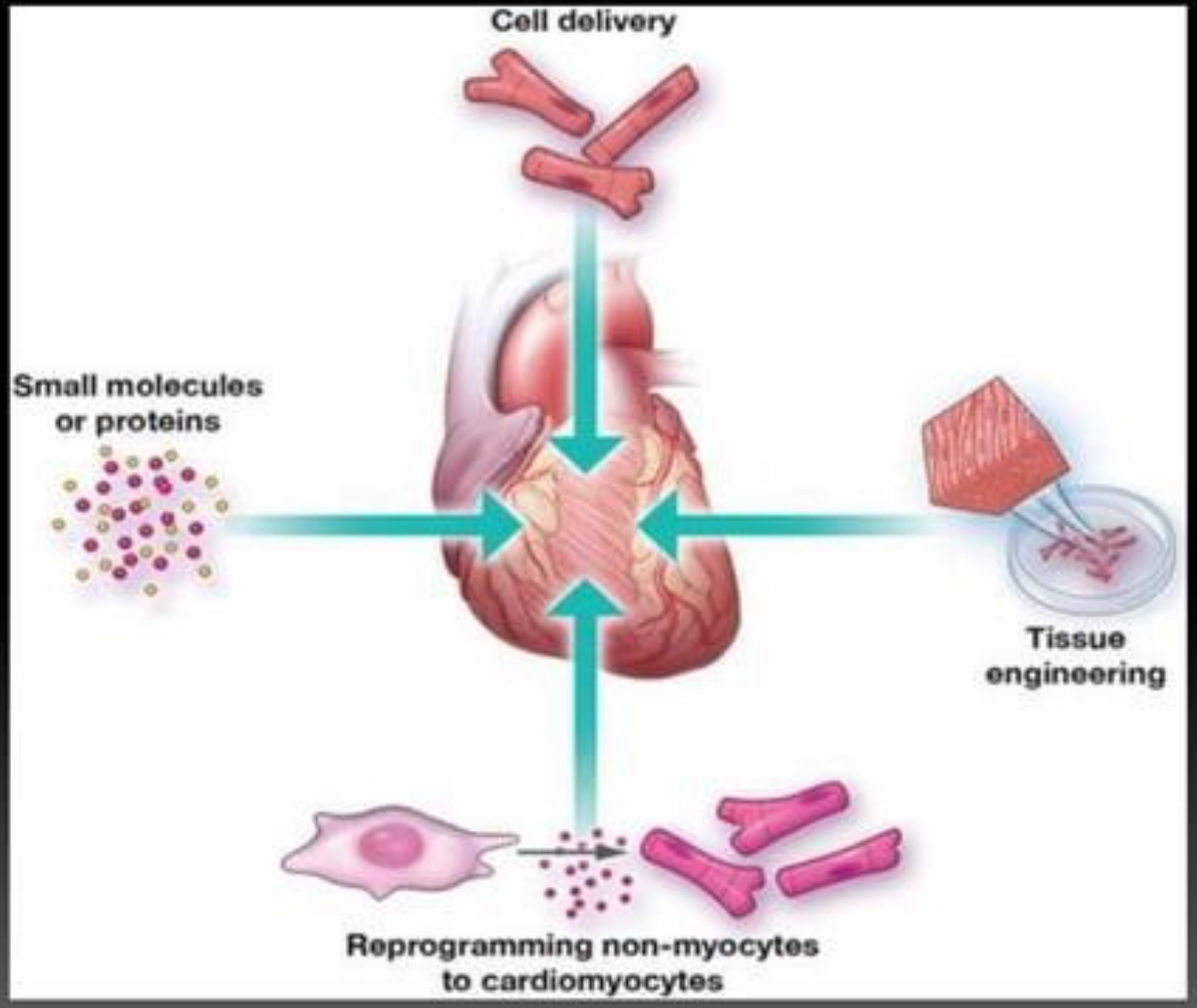
Stem cell based therapy

For the first time, doctors were able to improve **heart function and reverse tissue damage in heart failure patients**, using stem cells taken from the patients' own heart tissue.



http://www.stemcellresearchnews.net/News/
https://sites.google.com/site/regeneratingcardiactissueheart/stem-cells-to-regenerate-cardiac-tissue-in-heart-failure

Strategies to repair the heart

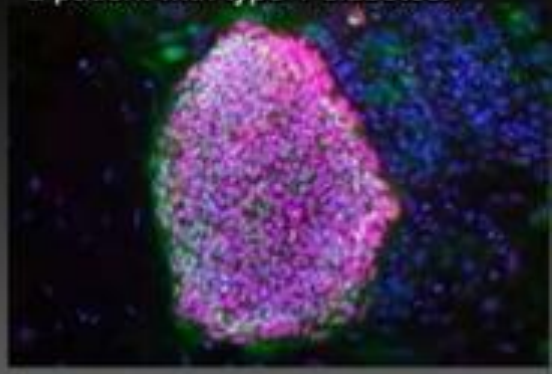


Cell Stem Cell 12, June 6, 2013 ª2013

Latest stories

- Embryonic stem cells, from a woman with Type 1 diabetes, were induced to turn into insulin-making beta cells, in hopes they could be implanted to cure the disease.
- Stem cells in the adult pancreas have been identified that can be turned into insulin producing cells, a finding that means people with type 1 diabetes might one day be able to regenerate their own insulin-producing cells.
- Ovary Stem Cells Can Produce New Human Eggs
- Clinical trial using stem cells in patients with amyotrophic lateral sclerosis

Insulin, shown in red, is being produced by cells that started as embryonic stem cells derived from a patient with type 1 diabetes.



PLoS ONE, 2012; 7 (11): e48977

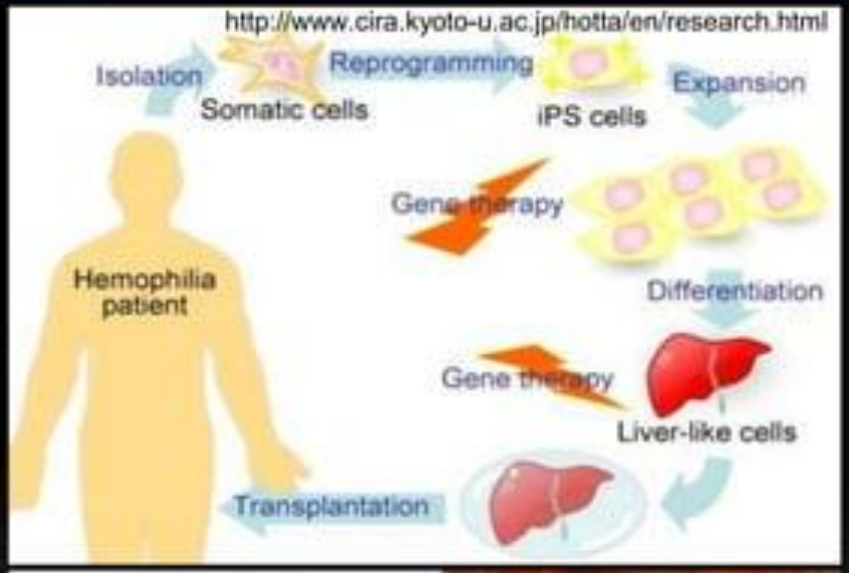
http://www.technologyreview.com/news/526911/stem-cells-from-a-diabetes-patient/

Clinical needs of the hour

- Disease reversal
- Treatment of disease
- Organ/tissue availability
- Aging
- Gene correction
- Drug delivery

http://abcnews.go.com/Health/experimental-stem-cell-therapy-burn-victims/story?id=17350731

http://www.cira.kyoto-u.ac.jp/hotta/en/research.html



www.smilemakeoverofla.com/bioteeth-clinical-use-of-stem-cell-research/

Stem cell and Bioengineering

- Production of Soluble Chemical Factors like EGF, FGF, TGF-β etc

Transduce signals
- Growth, Survival, Motility and Differentiation
- Cell type-dependent
- Differentiation stage-dependent

- Scaffold

Temporary structural support
- Maintain shape
- Surface coating and chemistry
- Rigidity, Porosity, topography

Cellular microenvironment
- High surface area/volume
- ECM secretion
- Integrin expression
- Facilitate cell migration

Stem cell and Bioengineering

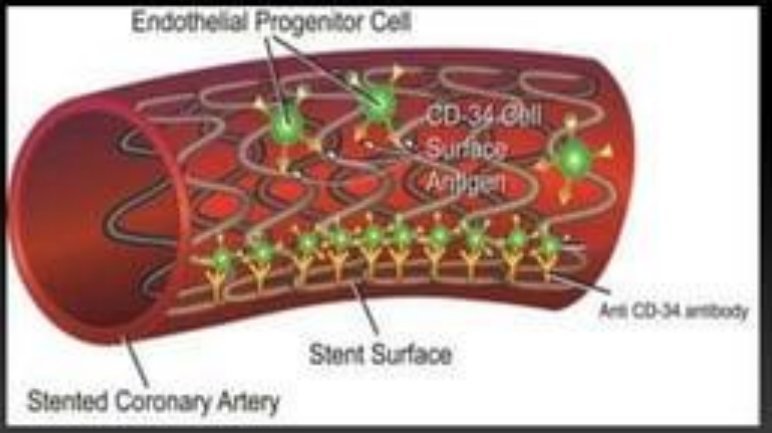
- Delivery Methods

  Injectable stem cells

  - Cells or cell-polymer mix
  - Adopt shape of environment
  - Controlled growth factor release

  Solid scaffold manufacturing

  - Computer-aided design and matching
  - Identifying defect shape



Stem cell Debate

Politics and Arguments

- In Aug, 2001, The Bush Government announced federal funds could only be used to study human embryonic stem cell lines that already existed.
- Stem cell research done with the use of private funding.
- The UK in 2001 made it legal to create cloned human embryos for use in medical research.
- President Barack Obama lifts 2001 restrictions on federal funding for human embryonic stem cell research.
- Different countries have different rules.
- Scientist can only use eggs from fertility clinics that do not fertilize when mixed with sperm.
- In Asia, South Korea, Singapore are all moving forward on stem cell research. Singapore allows therapeutic cloning but banned reproductive cloning.

India and Stem cell

The Indian Department of Biotechnology, together with the Indian Council of Medical Research, drafted the nation's stem cell policy in 2007, the Guidelines for Stem Cell Research and Therapy. India, Department of Biotechnology and Indian Council of Medical Research, *Guidelines for Stem Cell Research and Therapy*, November 2007, http://www.icmr.nic.in/stem_cell/stem_cell_guidelines.pdf.

The Guidelines call for the establishment of a national body for the review of stem cell research proposals, the National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT). This committee was established only recently, with the twelve-member group being formed by the government in March 2011.

The Guidelines divide research on human stem cells into three areas: permissible, restricted, and prohibited. Permissible research includes *in vitro* studies on previously established cell lines from any cell type (including ES cells), *in vivo* studies in animals with established cell lines from any type of stem cells (including ES cells), the establishment of new ES cell lines from "spare" IVF embryos, and clinical trials with minimally manipulated cells.

Ethical Arguments

Ethical arguments
The use of human embryoes

IS AN EMBRYO HUMAN?

'Lines that Divide'

Ethical Arguments….Lets discuss!!

- Is it morally right?
- Why do the embryos have to be destroyed for stem cell research? Isn't this the same as taking a life?
- Will embryo farms will be around the corner?
- Will organs be produced as per your specification?
- Why do we need to keep using embryos in research when we have new iPS cells?
- Could women be forced to sell eggs/ embryos for research?
- Won't doing therapeutic cloning lead to cloning humans?
- **ARE HUMAN PLAYING GOD?**

Science is discovering the unknown

- Stem cell field is still in its infancy
- Human embryonic stem cell research is a decade old, adult stem cell research has 30-year head start
- Holds hope for curing or improving treatments for 70+ diseases
- Veterinary applications need to be thought about

How can you help to shape the direction of this field?

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THANK YOU