STEM CELLS

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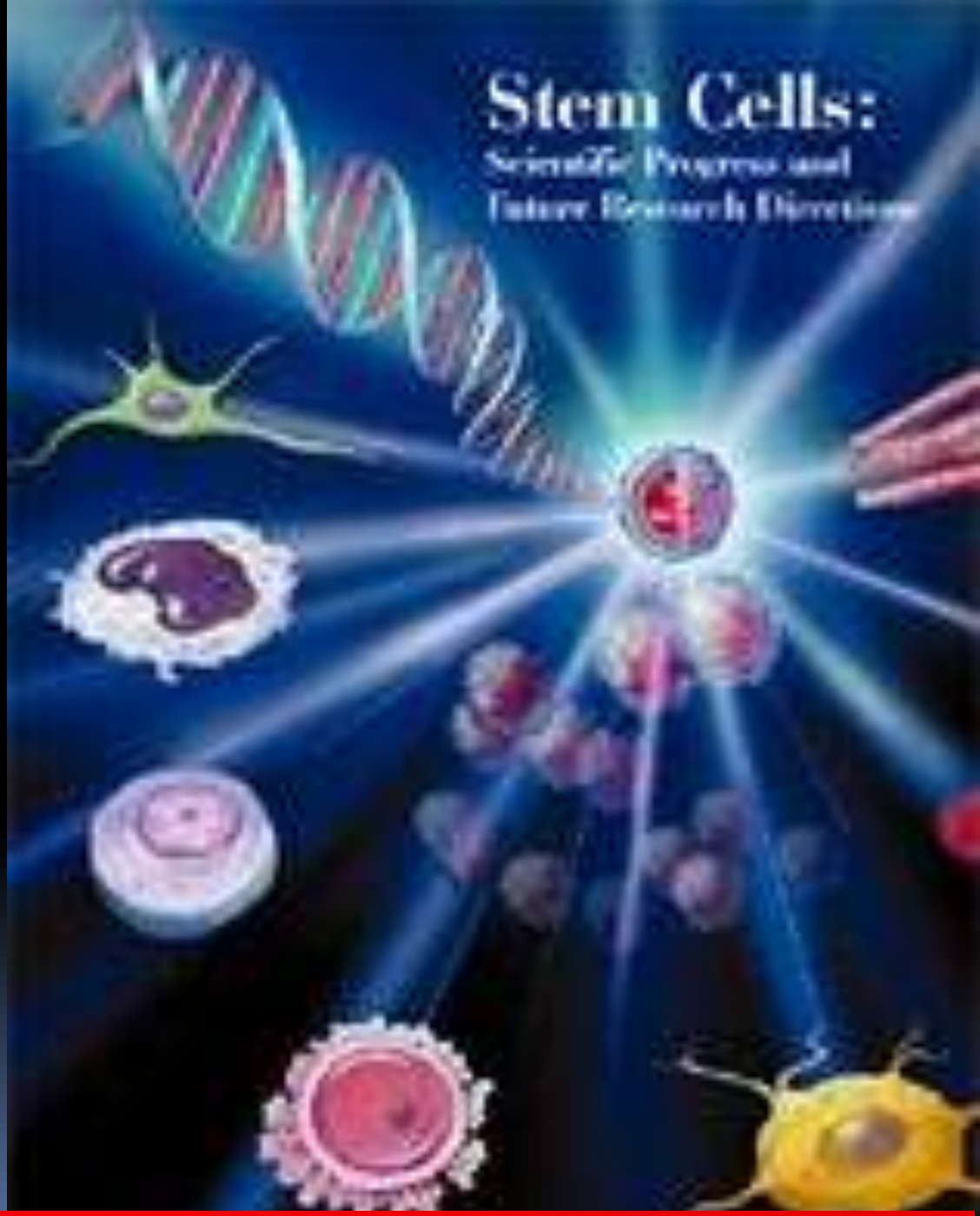
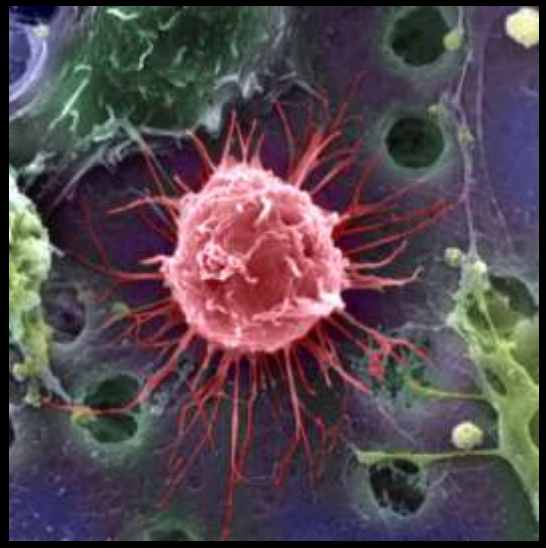
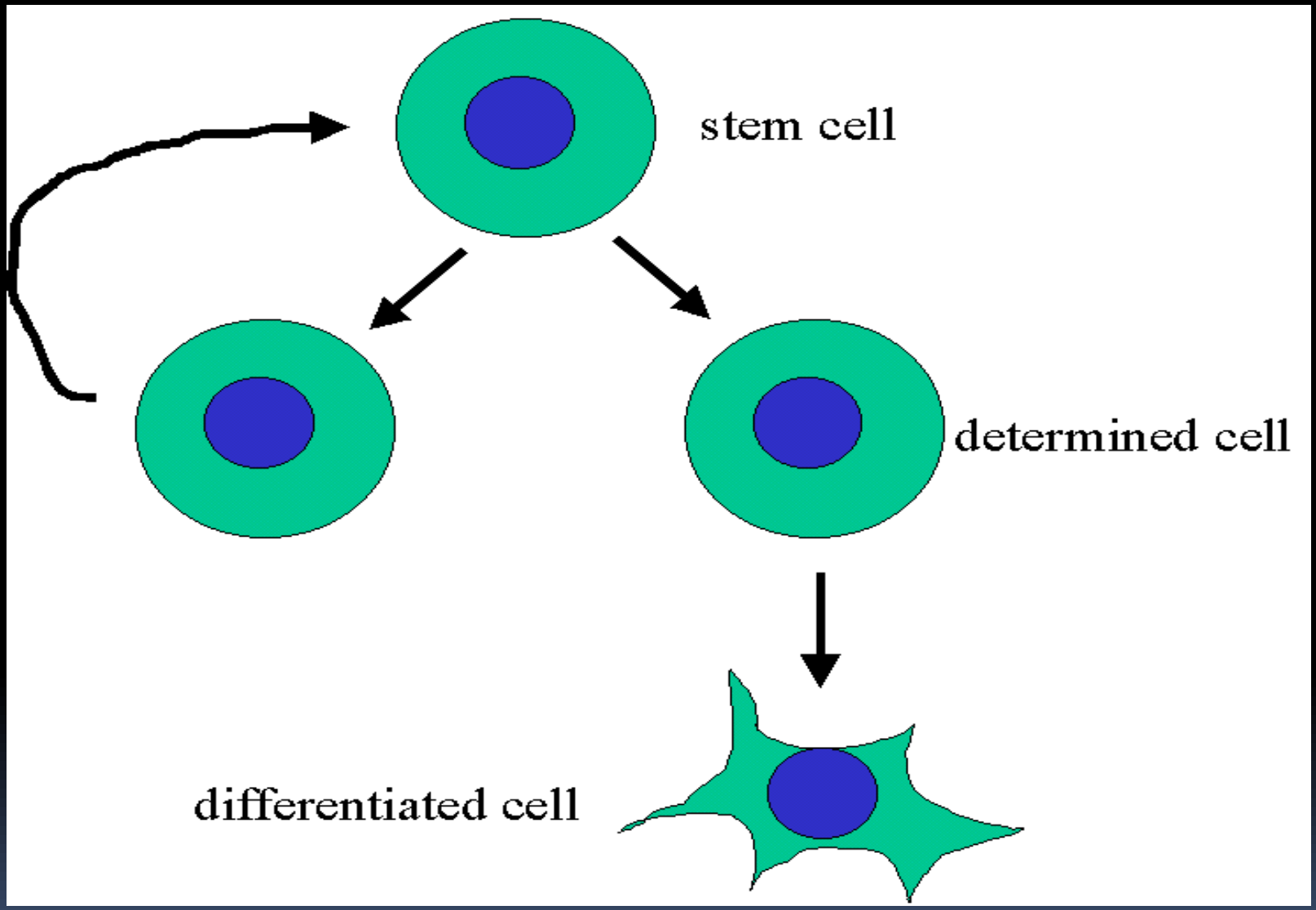


Introduction:



- stem cells:
  - (i) renew itself indefinitely
  - (ii) give rise to multiple tissue types

- A stem cell is not committed to a specific function until it receives a signal to differentiate into a specialized cell


stem cell
determined cell
differentiated cell

Benefits of stem cell research :

- Treatment of complex diseases
  - Diabetes
  - Alzhimer’s
  - Parkinson’s
  - Heart disease
  - Cancer

- Regenerative medicine (Spare parts !)
  - Skin
  - Cartilage
  - Bone

- Understanding human development

- Cell lines for research

Types of Stem Cells:

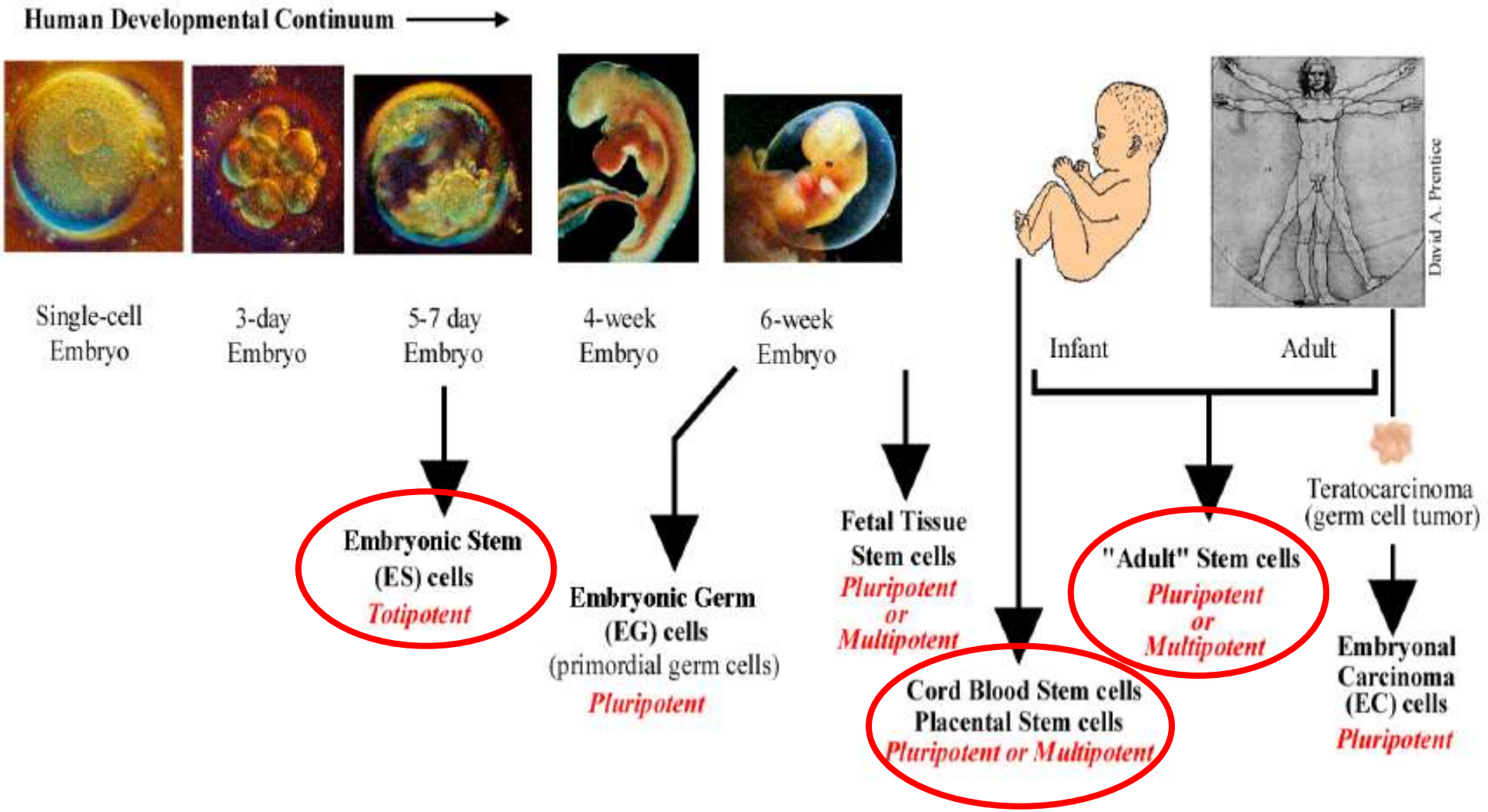
1. **Embryonic:**
   - Blastomere (4-5 day embryo)
   - Pluripotent

2. **Adult:**
   - Adult tissue
   - multi or uni potent

Other :

- **Fetal:**
  - Aborted embryos
  - Pluripotent
- **Umbilical:**
  - umbilical cord blood
  - multipotent

Stem Cells


Human Developmental Continuum
Single-cell Embryo
3-day Embryo
5-7 day Embryo
4-week Embryo
6-week Embryo
Infant
Adult
David A. Prentice
Embryonic Stem (ES) cells
Totipotent
Embryonic Germ (EG) cells
(primordial germ cells)
Pluripotent
Fetal Tissue Stem cells
Pluripotent or Multipotent
Cord Blood Stem cells
Placental Stem cells
Pluripotent or Multipotent
"Adult" Stem cells
Pluripotent or Multipotent
Teratocarcinoma
(germ cell tumor)
Embryonal Carcinoma (EC) cells
Pluripotent

Stem cells and Potency:

1.Totipotent (Fertilized egg)

Generate: - all embryonic cells and tissues
- supporting tissue like placenta and umbilical cord

2. Pluripotent stem cells

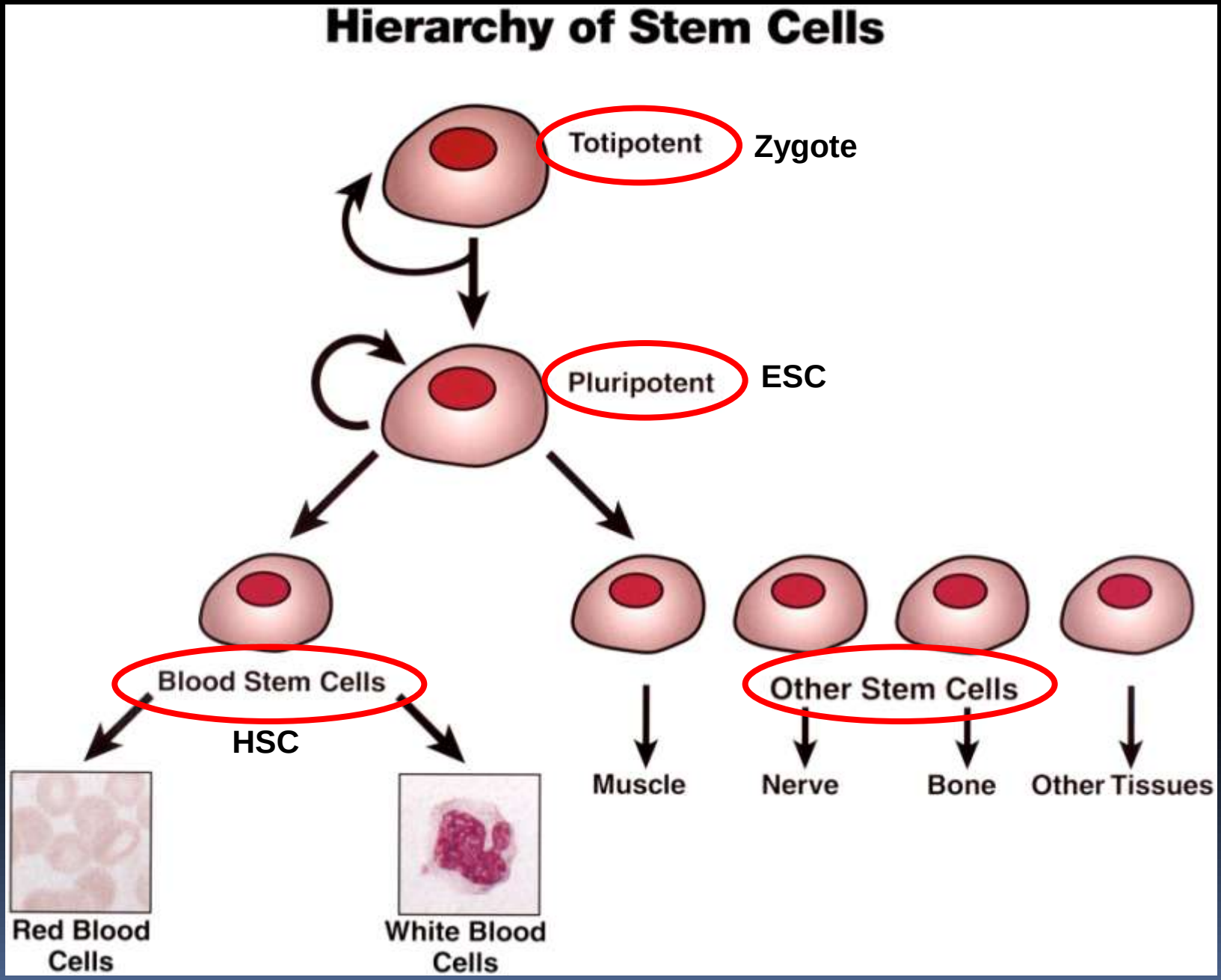
- Give rise to cells of all 3 germ layers (ecto-, meso-, and endoderm
- Come from embryos and fetal tissue.
- Have active telomerase (maintain long telomers).

3. Multipotent Stem cells

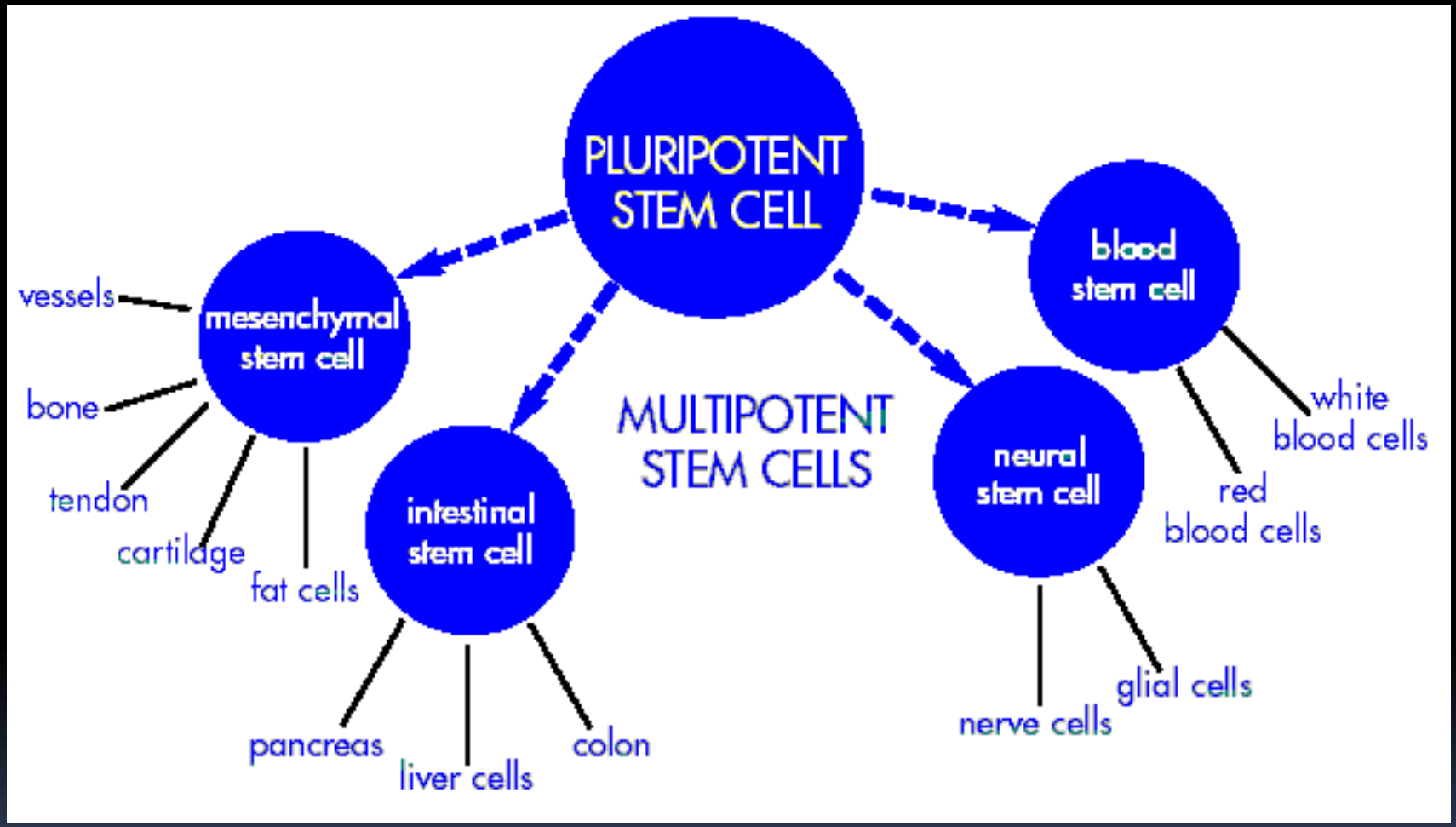
- Give rise to multiple different cell types.

4. Unipotent stem cell

- Found in adult organism
- Cell differentiating along only one lineage


Hierarchy of Stem Cells
Totipotent
Zygote
Pluripotent
ESC
Blood Stem Cells
HSC
Other Stem Cells
Muscle
Nerve
Bone
Other Tissues
Red Blood Cells
White Blood Cells

DIFFERENTIATION POTENTIAL OF STEM CELLS



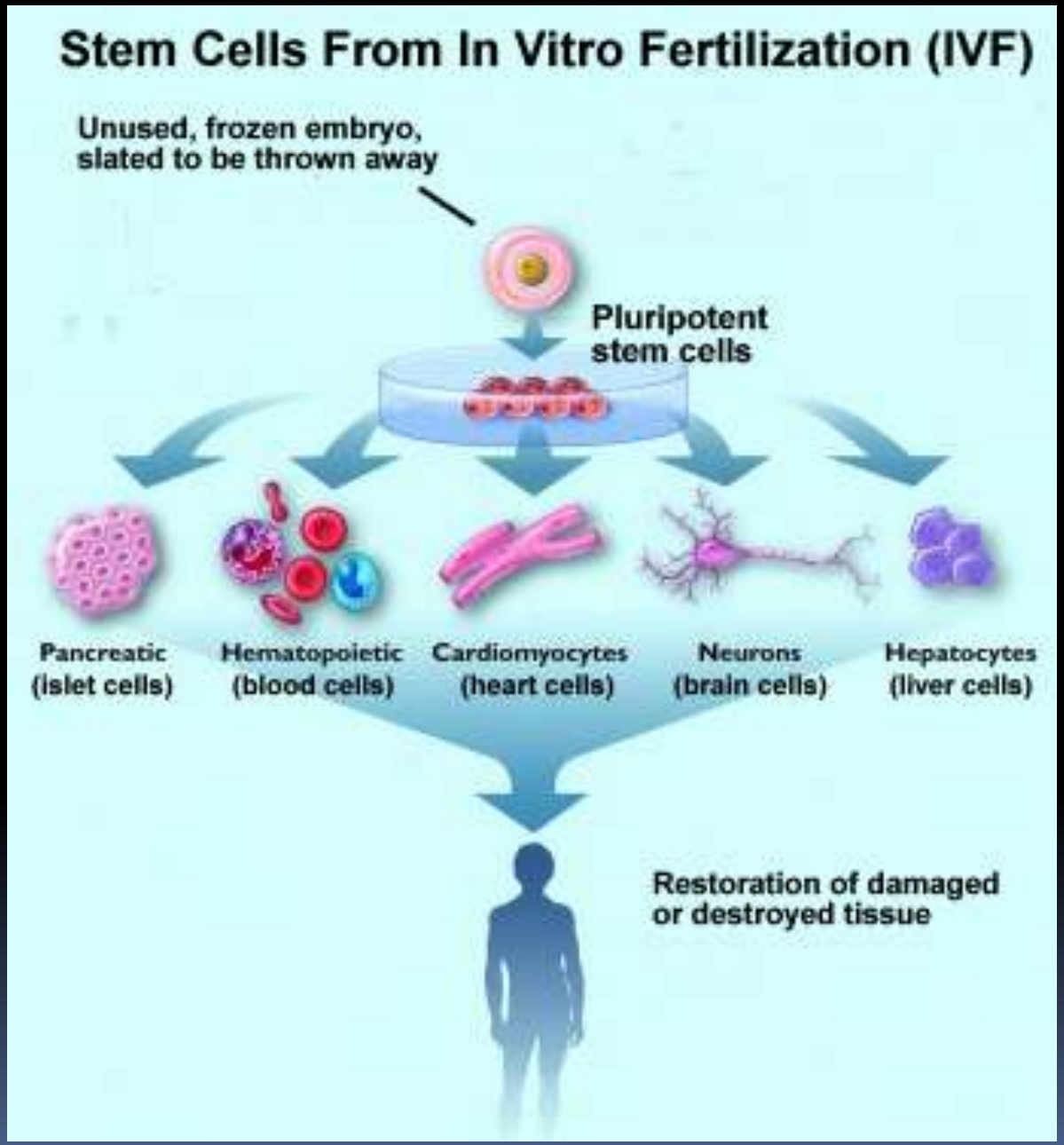
The Embryonic Stem Cell

Source:

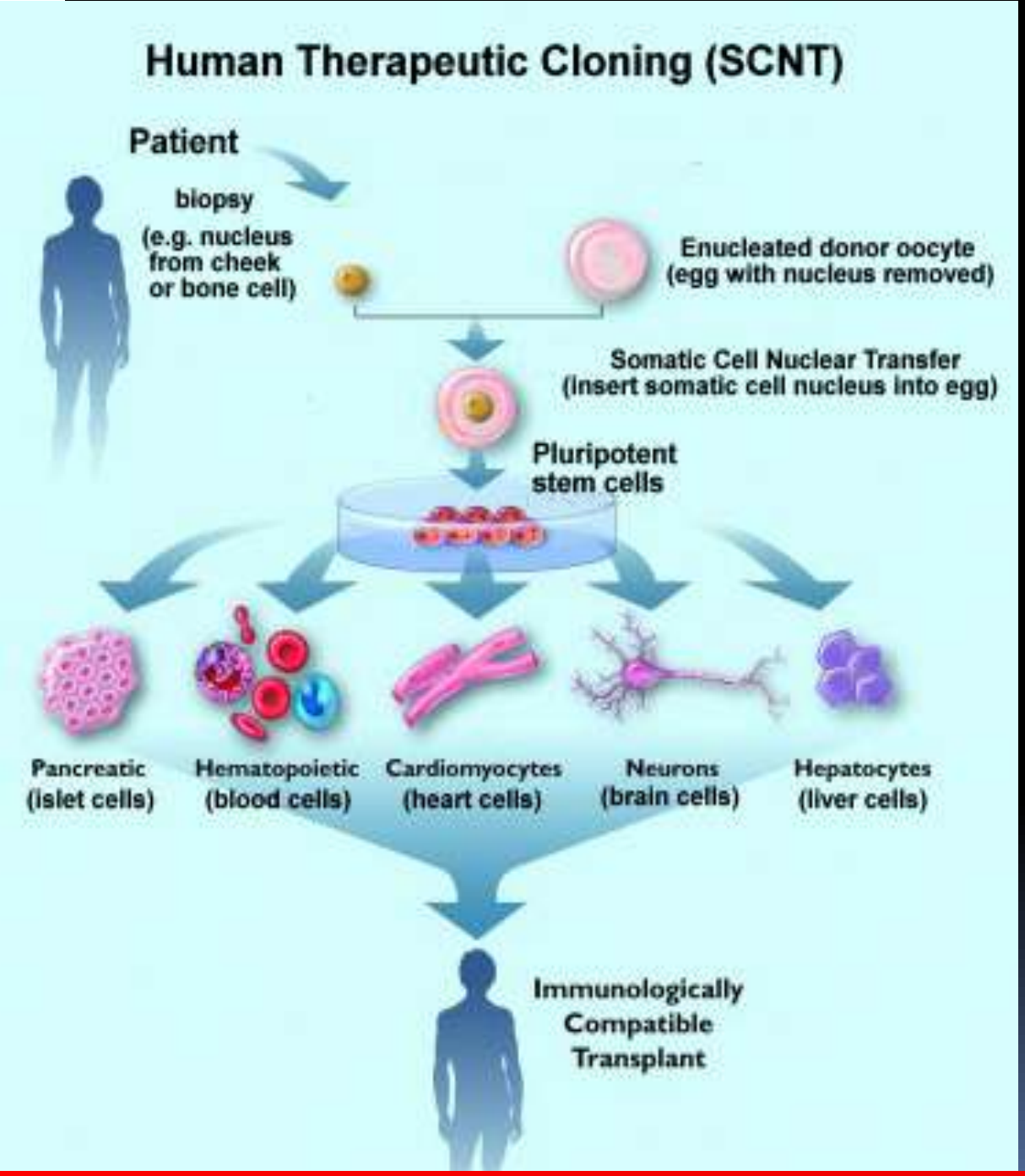
1. IVF embryos

2. Aborted Fetus

3. Therapeutic cloning



Tens of thousands of frozen embryos are routinely destroyed when couples finish their treatment.



Somatic Cell Nuclear Transfer

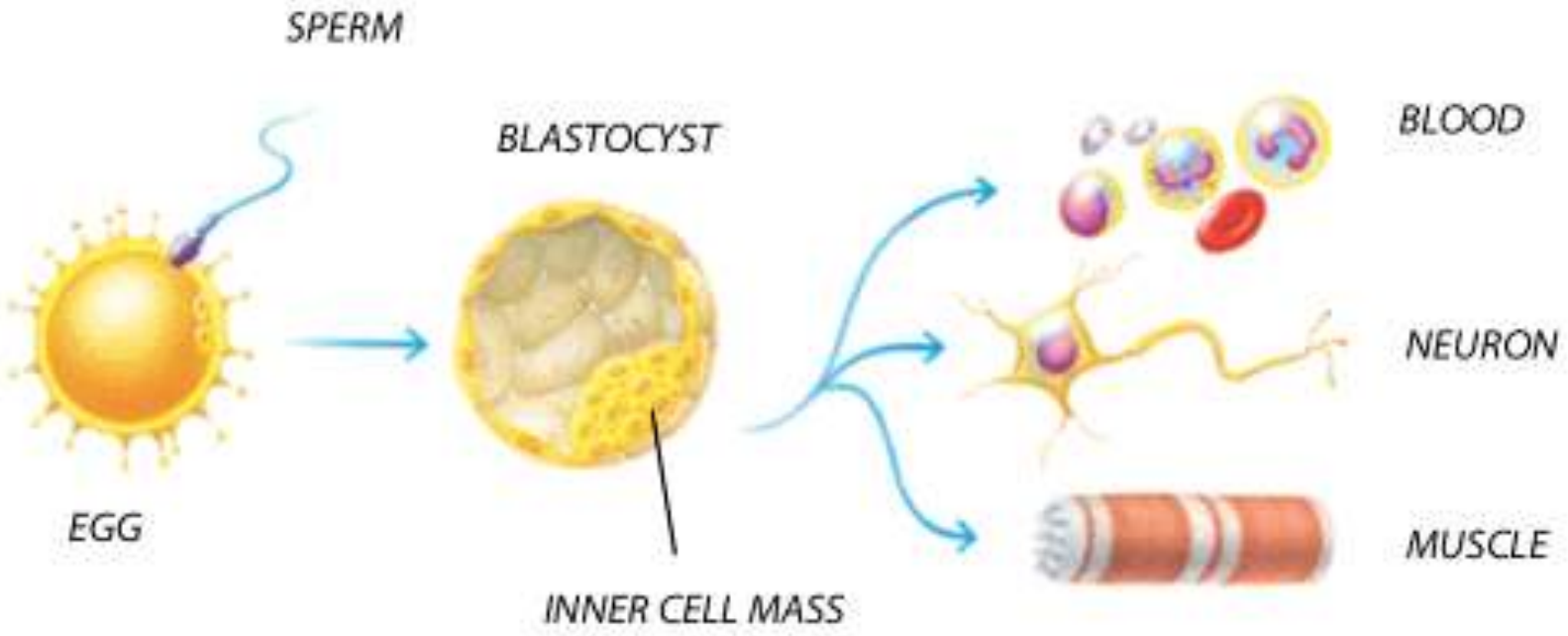
The nucleus of a donated egg is removed and replaced with the nucleus of a mature, "somatic cell" (a skin cell, for example).

No sperm is involved

Embryonic Stem Cell

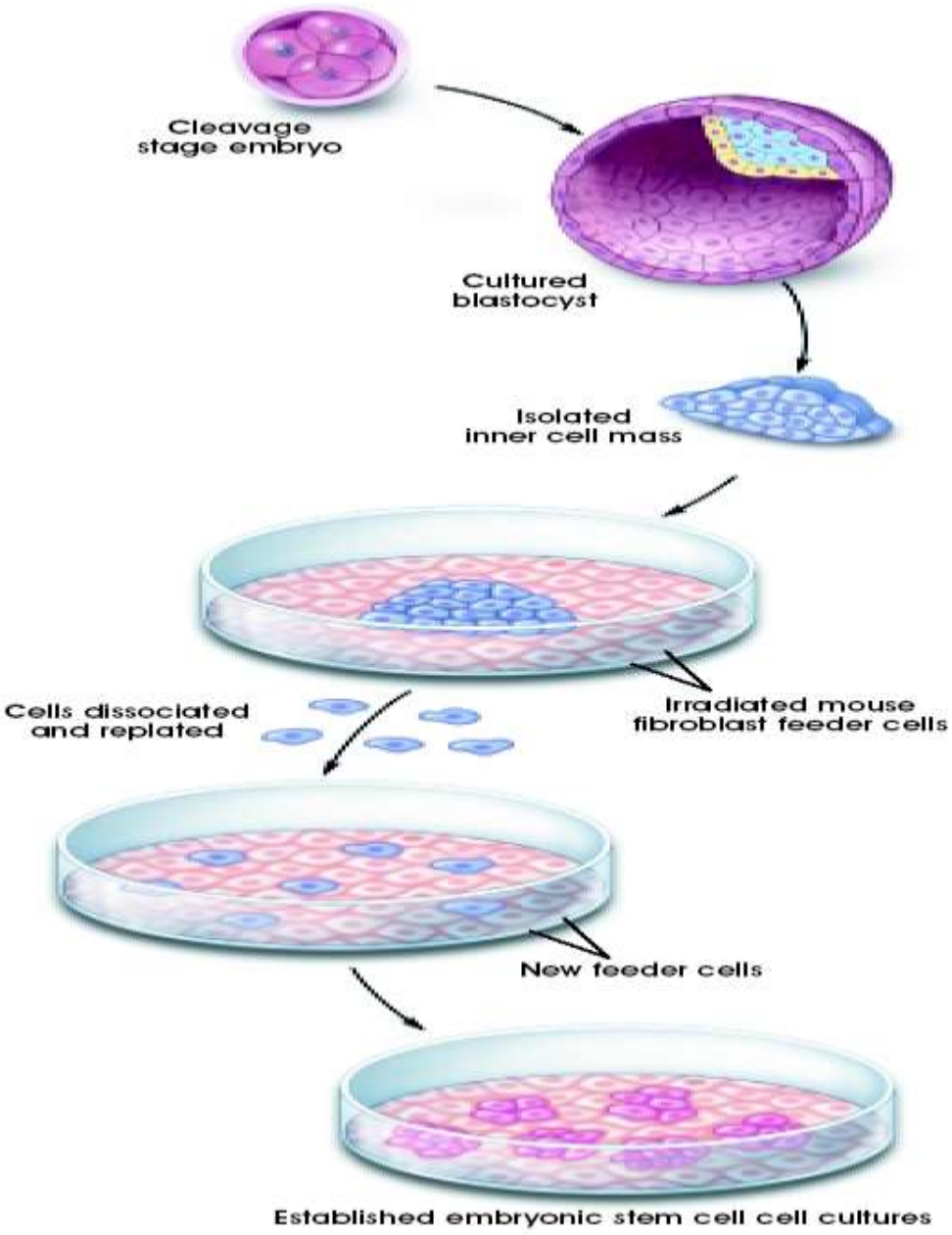
- First isolated and cultured in 1998
- From inner cell mass of blastocyst (4-5 day embryo).
- Pluripotent with long-term self-renewal
- Capable of unlimited number of divisions without differentiation
- Can essentially live forever without forming tumors
- Maintain normal diploid complement of chromosomes (stable karyotype)
- Telomerase activity
- Clonogenic: give rise to genetically identical group of cells
- Expresses transcription factor Oct-4 (+ or – genes needed for proliferative state)
- Spend most of their time in S phase
- *In-Vitro: 300* population doublings

origin of Embryonic stem cell



SPECIALIZATION: Some scientists consider a stem cell to arise when a sperm fertilizes an egg (left). Others think it originates in the inner cell mass of the blastocyst, a hollow sphere that emerges after several cell divisions (center). Either way, stem cells can differentiate into unique cell types, such as muscle, blood and nerve tissue (right).

GROWING HESC IN VITRO:



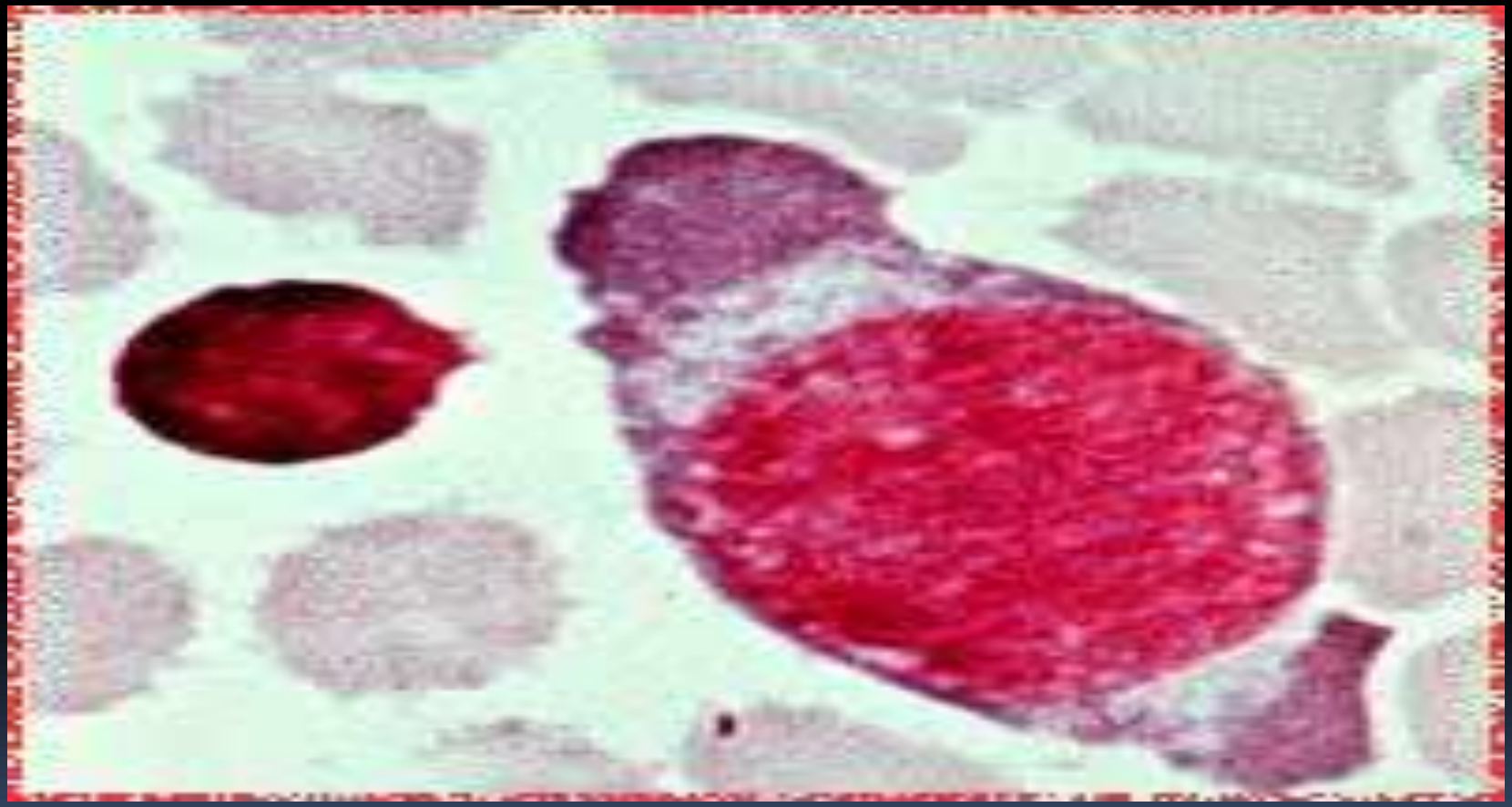
Advantages:

- Immortal: supply endless amount of cells
- Flexible: can make any body cell
- Available: IVF clinics

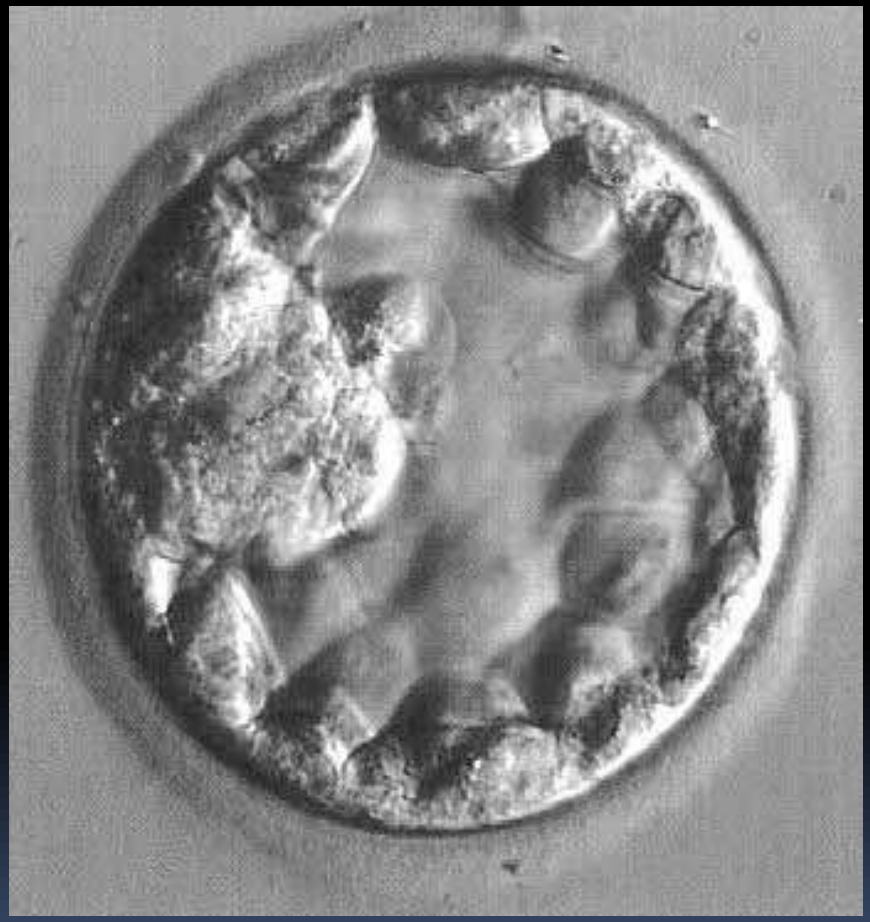
Disadvantages:

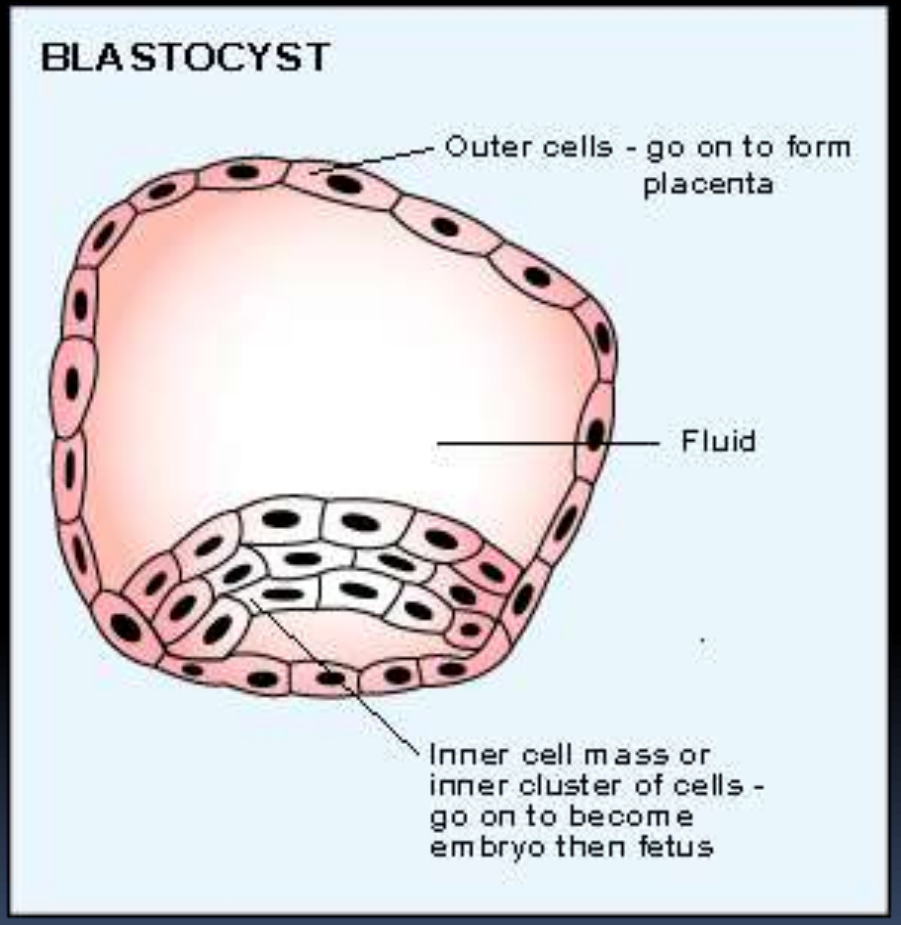
- Hard to control their differentiation
- Ethics
- Immune rejection

Embryonic stem cells



Human **Blastocyst** showing Inner Cell Mass





Embryonic Stem Cell

Nerves

Blood

Liver & Pancreas

Tissues

Vascular Systems

Bone

Muscle

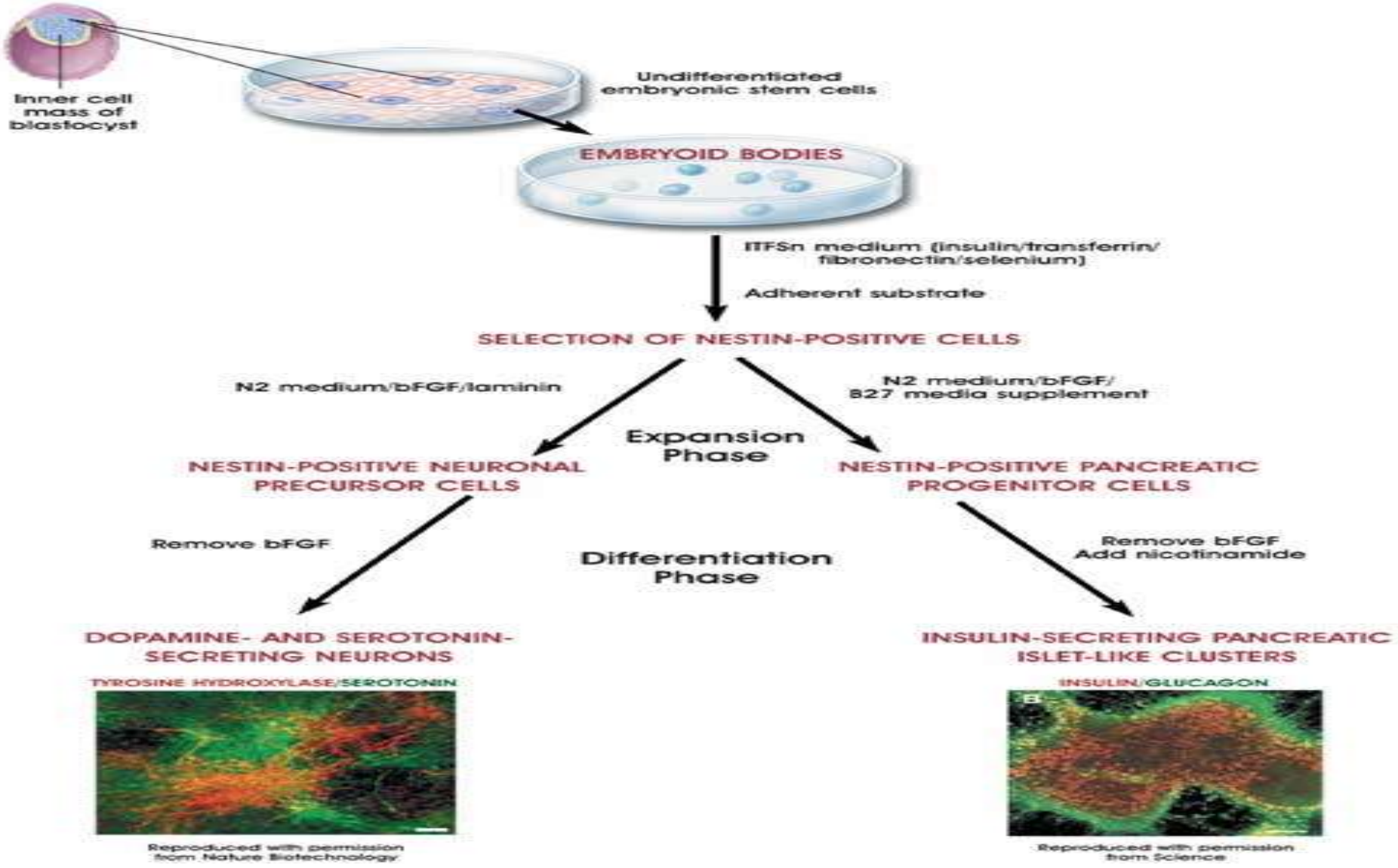
Target Discovery

Target Validation

Customized Assays

Drug Lead Prioritization (optimization)

Directed differentiation of mouse embryonic stem cells



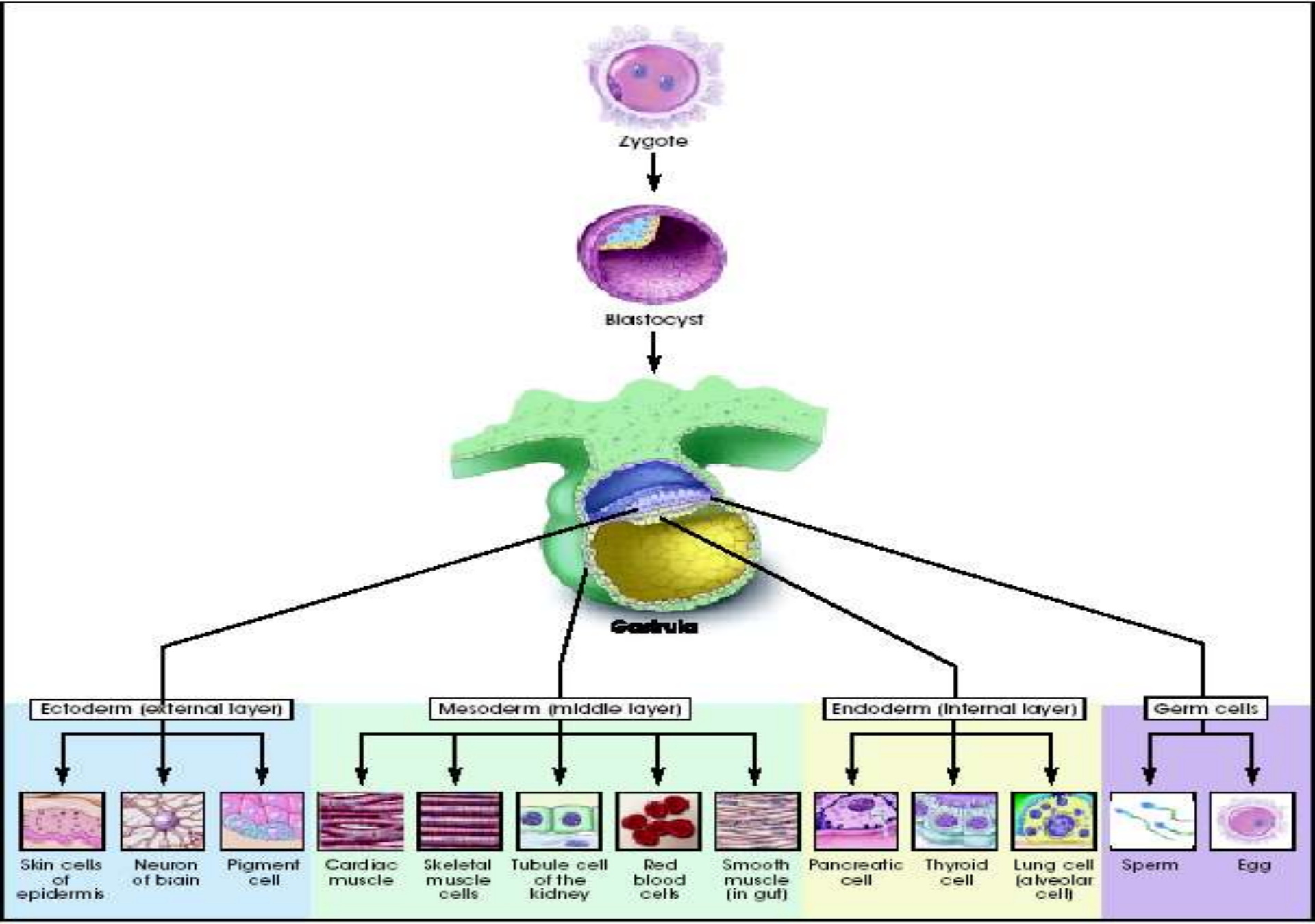


Figure 1.1. Differentiation of Human Tissues.

Human embryonic germ cell:

- Derived from fetal tissue.

- Primordial germ cells of gonadal ridge of 5 – 10 week fetus

- Primordial germ cells give rise to eggs or sperms.

- Pluripotent

-Stable karyotype

-*In-Vitro:* 70-80 population doublings

-Telomerase and telomere status not very clear

COMPARISONS BET. HESC & HEGC :

Similar in :

- Replicate for extended period of time.
- Generate both XX and XY cultures.
- Express markers of pluripotent cells.
- Differentiate into cells of 3 germ layers.

Differ in :

- Tissue sources
- Growth characteristics *in vitro*, and behavior *in vivo*.
- Population doublings ES (300), EG (70-80)
- Teratomas .. (ES generate, EG not)
- Active telomerase in ES (EG unknown)

Directing Differentiation of HESC & HEGC In-vitro:

Signals:

- **Cell death: death of near-by cells stimulates stem cells**
  (Stem cells injected in eyes of rats with damaged retina migrate towards damage area)

- **Chemical growth factors and signals:**
  (RA),(EGF),(BMP4),(bFGF)→ ectoderm
  Activin-A (TGF-ß1) → mesoderm
  (HGF), (NGF) → 3 germ layers, including endoderm.

Avoiding Immuno rejection:

1. Genetically engineering stem cell to:
   a. Express MHC antigens of recipient
   b. produces stem cells with deleted MHC genes

1. Therapeutic Cloning:
   Clone somatic Cell nucleus of recipient into egg
   develop into blastocyst and isolate ES cells
   Such ES cells have recipient immunological profile

3. Co-transplantation with Hematopoitic Stem cells

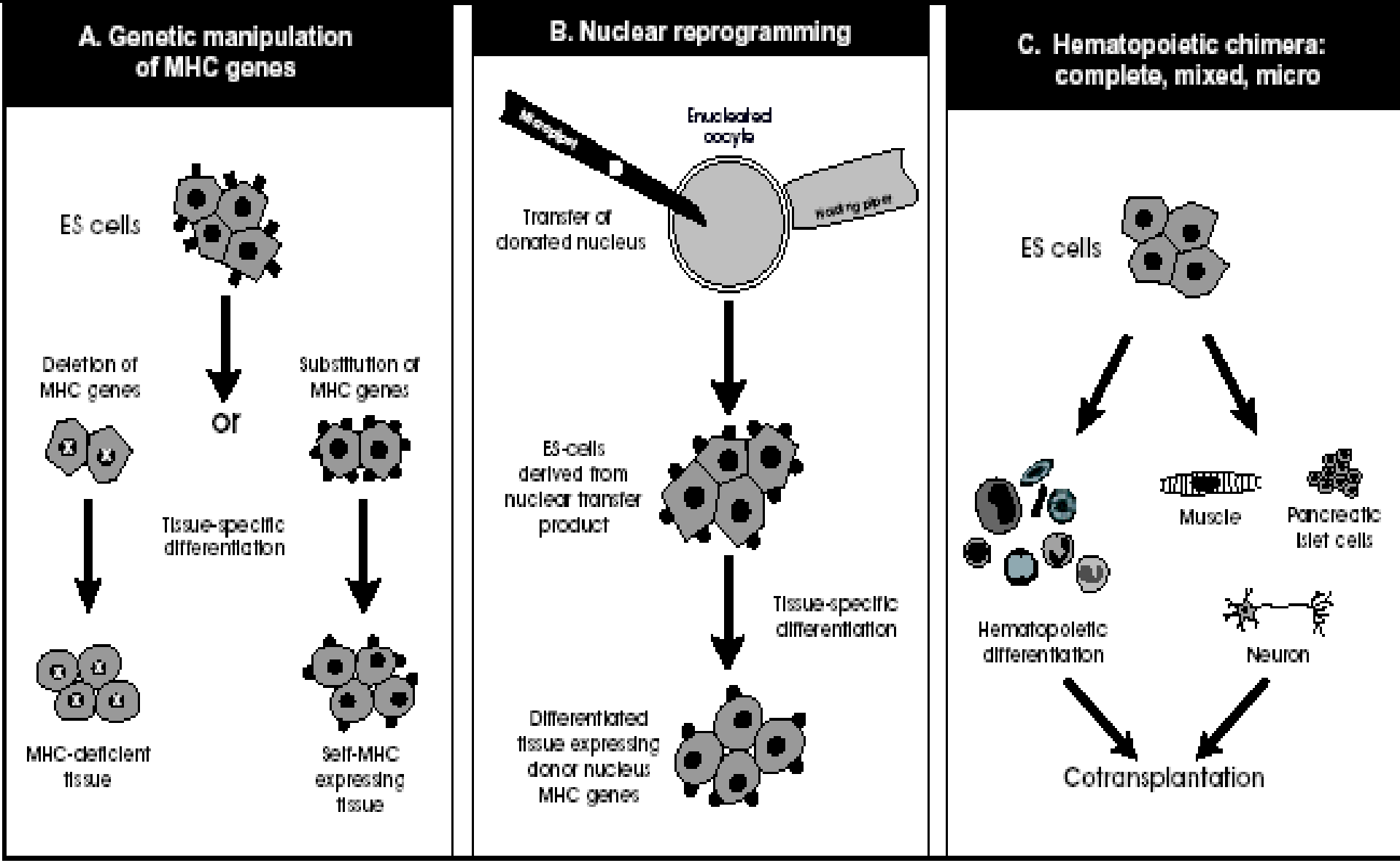


Figure 3.3. Genetic Manipulation of Human Embryonic Stem Cells. (Reproduced with permission from Stem Cells, 2001)

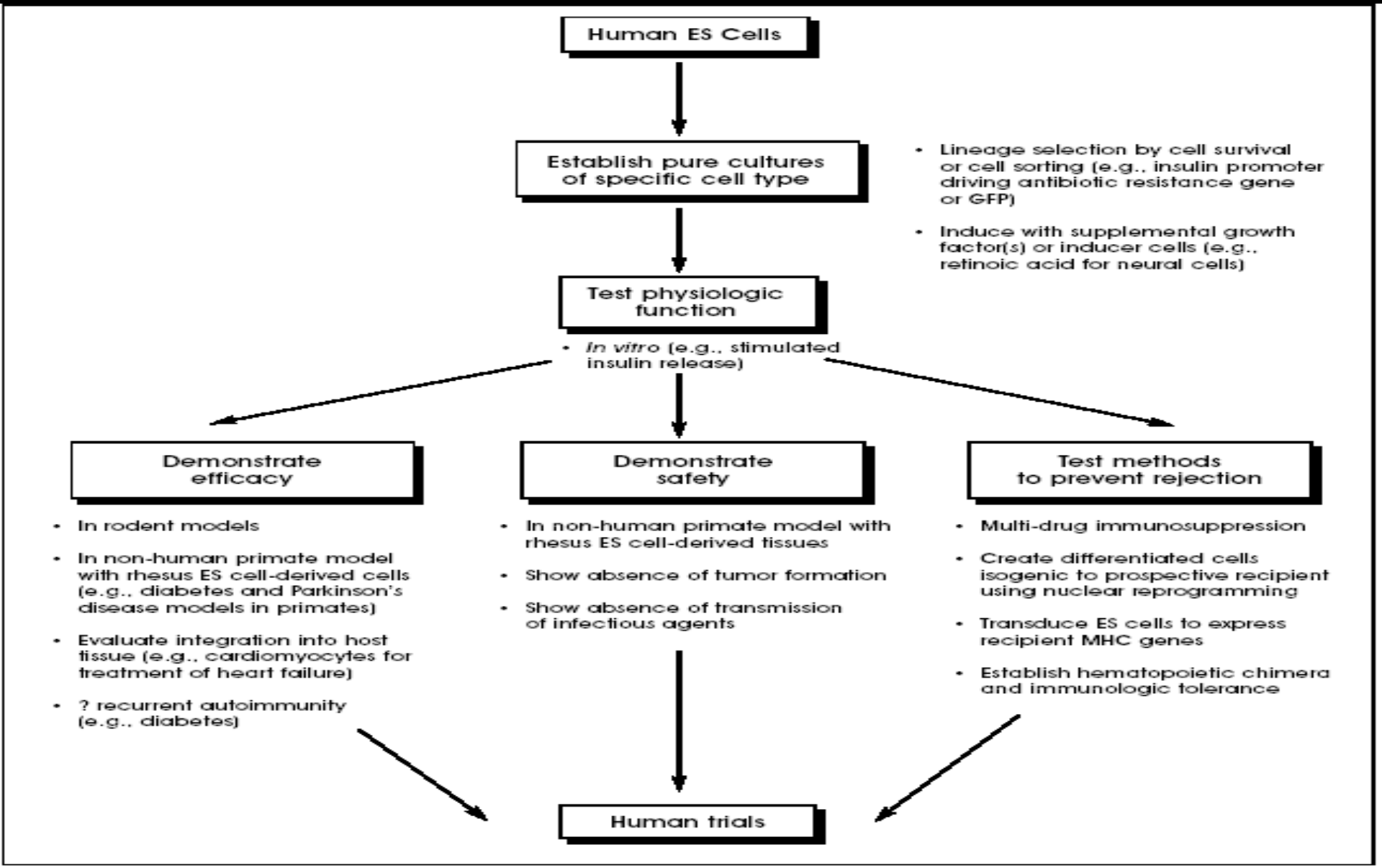


Figure 3.2. Major Goals in the Development of Transplantation Therapies from Human ES Cell Lines. (Reproduced with permission from Stem Cells, 2001)

Laboratory tests to identify ESC :

1. Sub-culturing stem cells for many months (long-term self-renewal)

2. Inspect cultures by microscope (for undifferentiation)

3. Determine surface markers & Test presence of Oct-4

4. Examining the chromosomes

5. Test ability to subculture the cells after freezing, thawing, and re-plating.

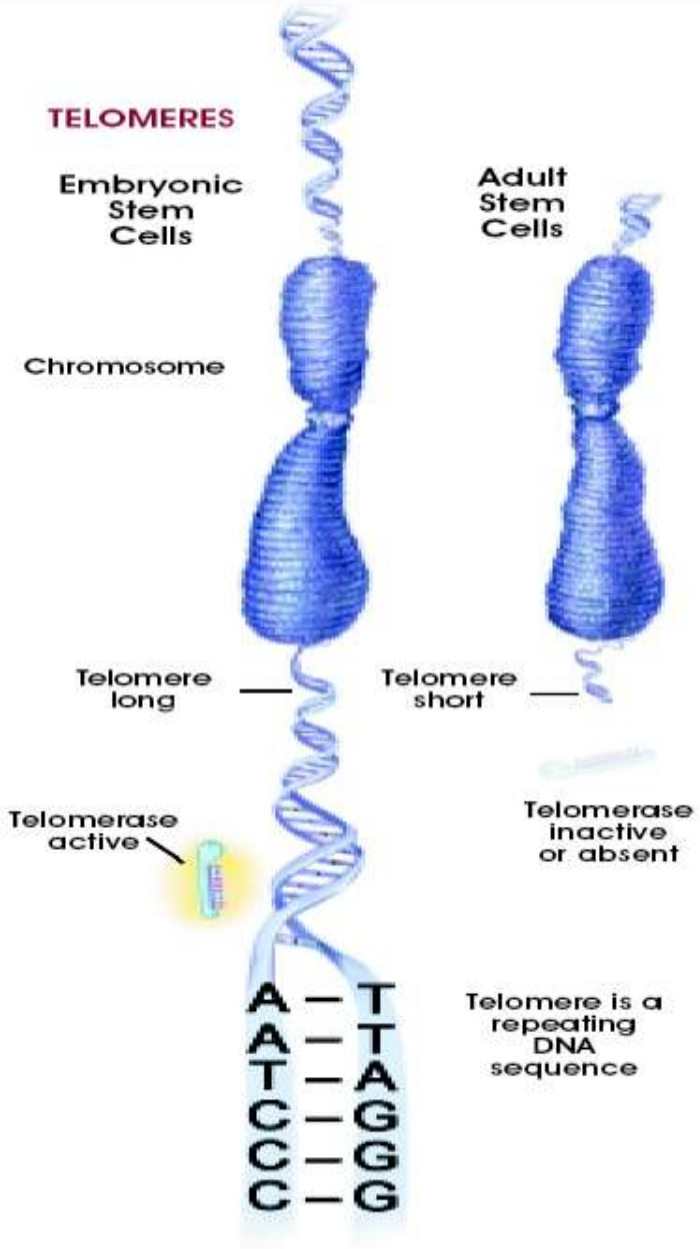
6. Test pluripotency by:

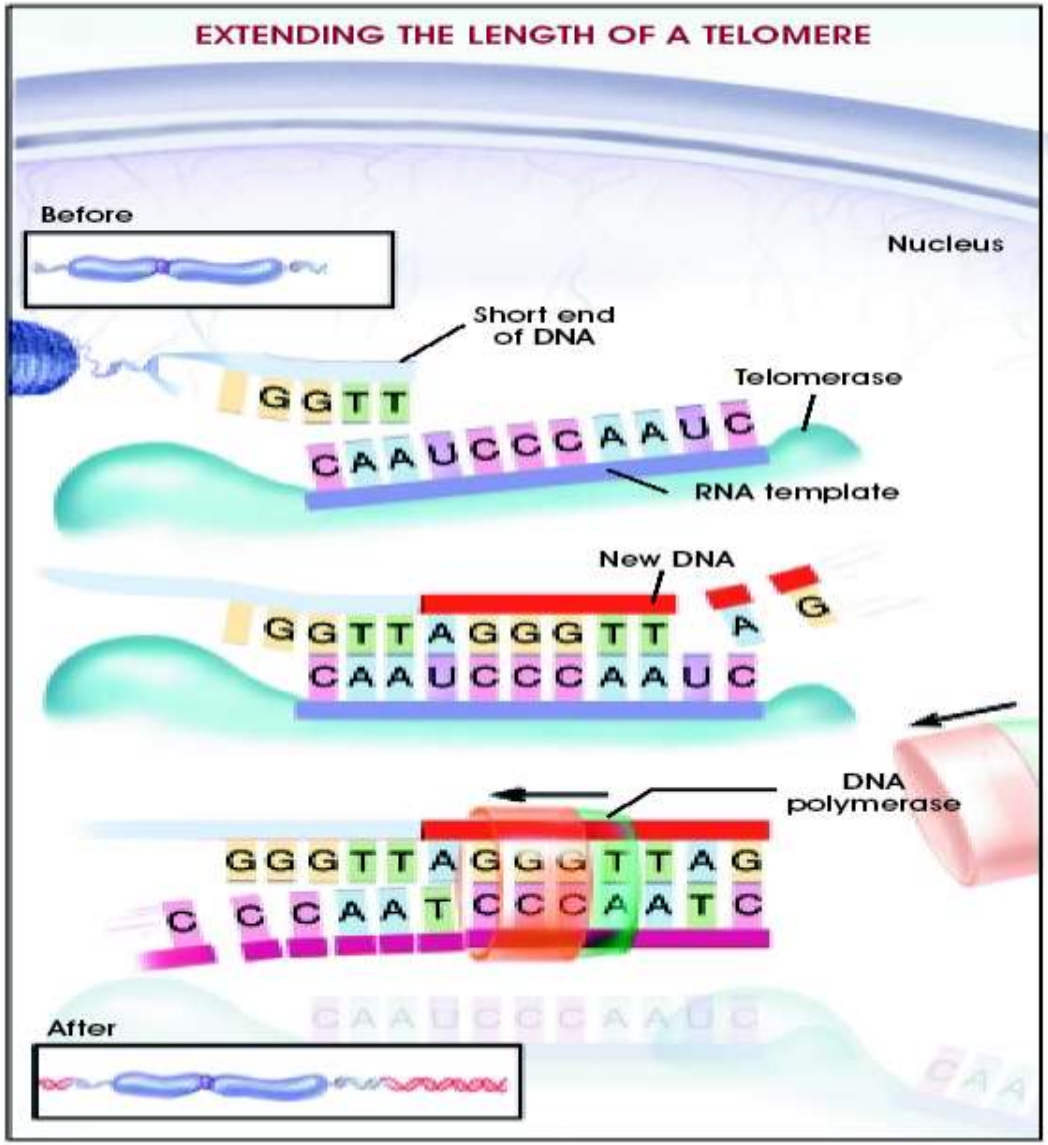
- Allowing cells to differentiate spontaneously in cell culture

- Manipulating the cells to differentiate to specific cell types

7. Test Telomerase activity and long telomeres in ESC

ES cell lines express high levels of telomerase





The Adult Stem Cell

- Undifferentiated cell found in a specialized tissue in adult.
- Capable of self-renewal
- Become specialized to cell types of the tissue from which it originated.

Properties:

- Somatic stem cell
- Long-term self-renewal
- give rise to mature cell types
- Generate intermediate cell (progenitors) “committed”
- Can migrate whenever needed
- No evidence adult stem cell is pluripotent

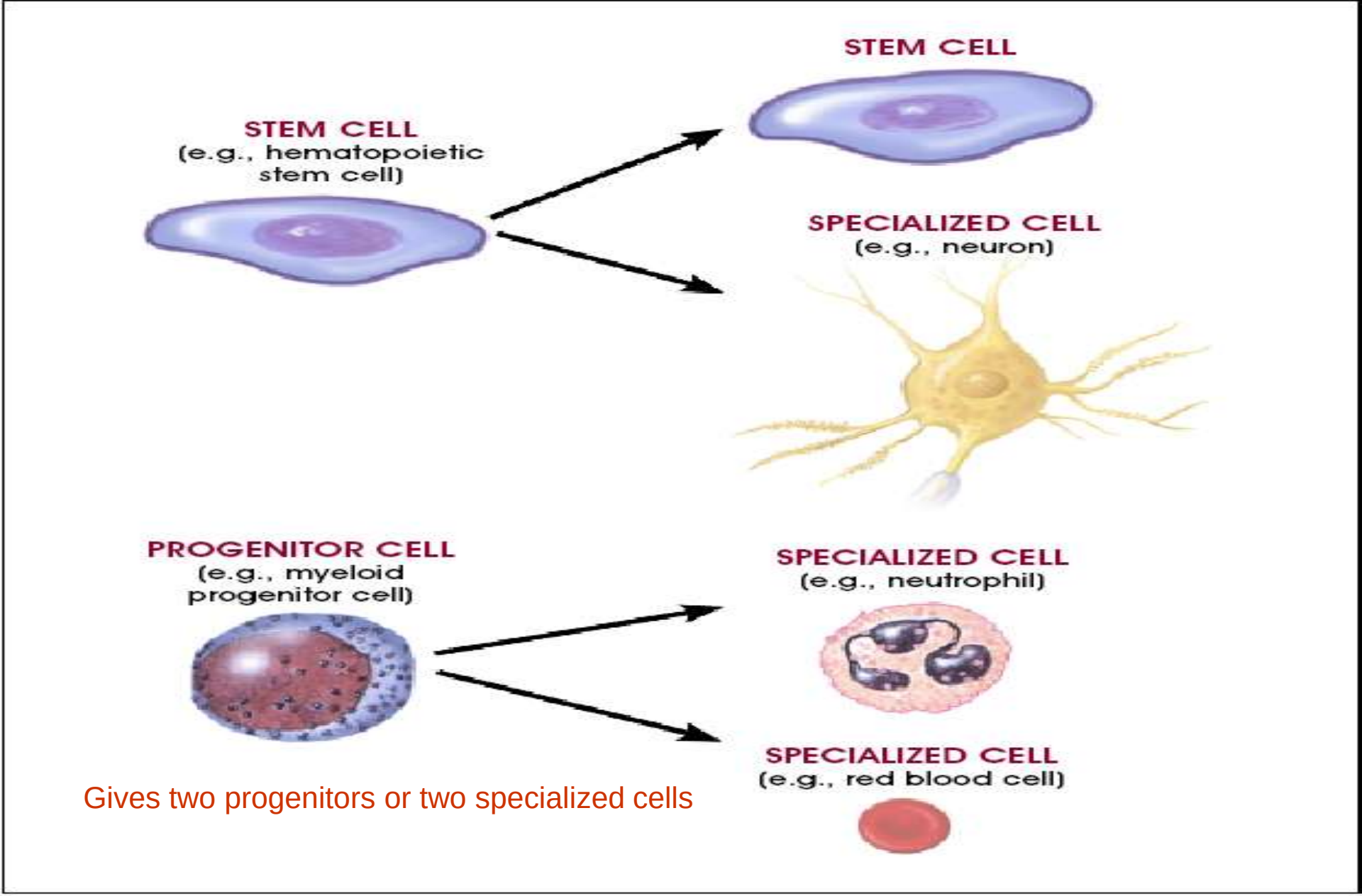
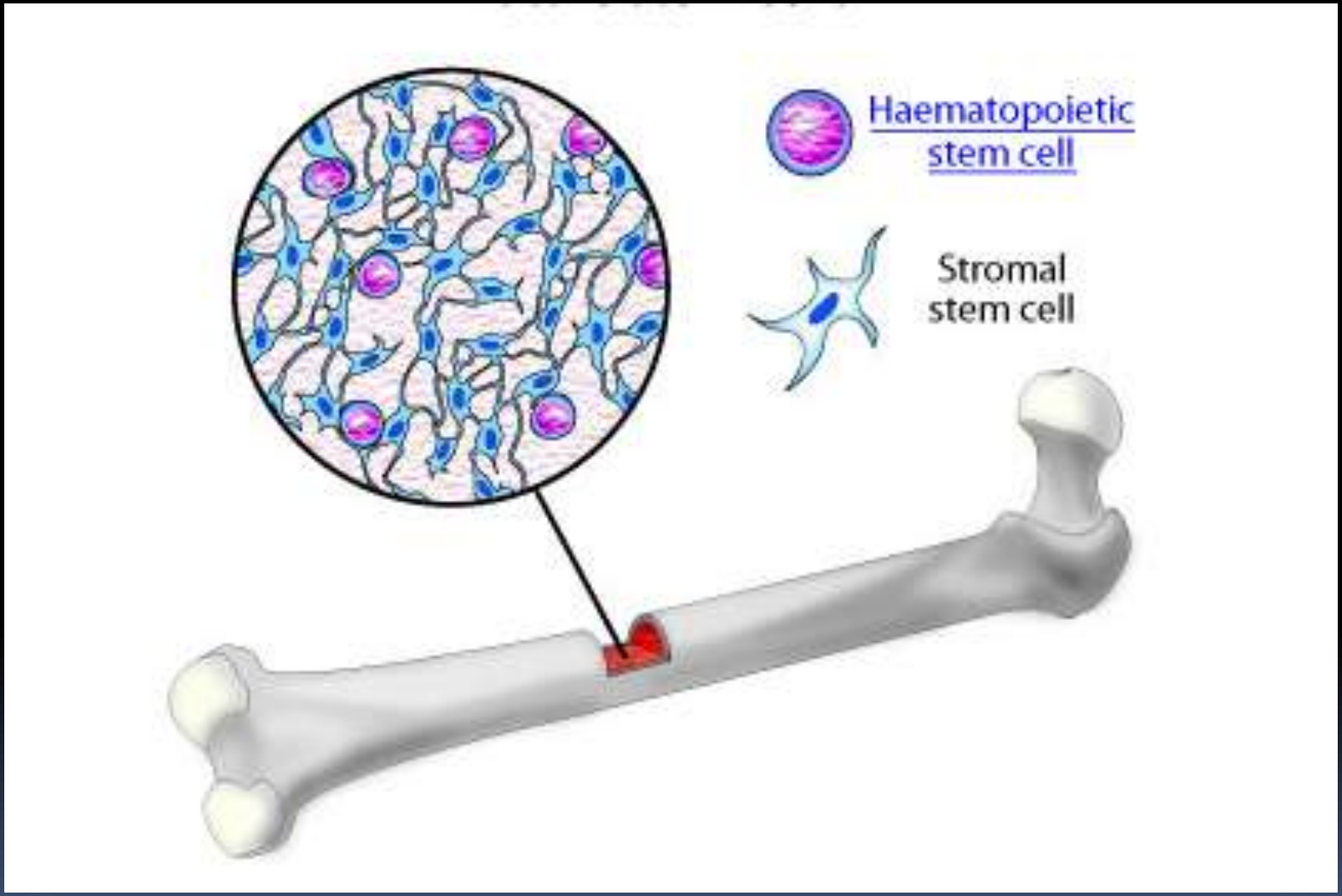


Figure 4.1. Distinguishing Features of Progenitor/Precursor Cells and Stem Cells. A stem cell is an unspecialized cell that is capable of replicating or self renewing itself and developing into specialized cells of a variety of cell types. The product of a stem cell undergoing division is at least one additional stem cell that has the same capabilities of the originating cell. Shown here is an example of a hematopoietic stem cell producing a second generation stem cell and a neuron. A progenitor cell (also known as a precursor cell) is unspecialized or has partial characteristics of a specialized cell that is capable of undergoing cell division and yielding two specialized cells. Shown here is an example of a myeloid progenitor/precursor undergoing cell division to yield two specialized cells (a neutrophil and a red blood cell).

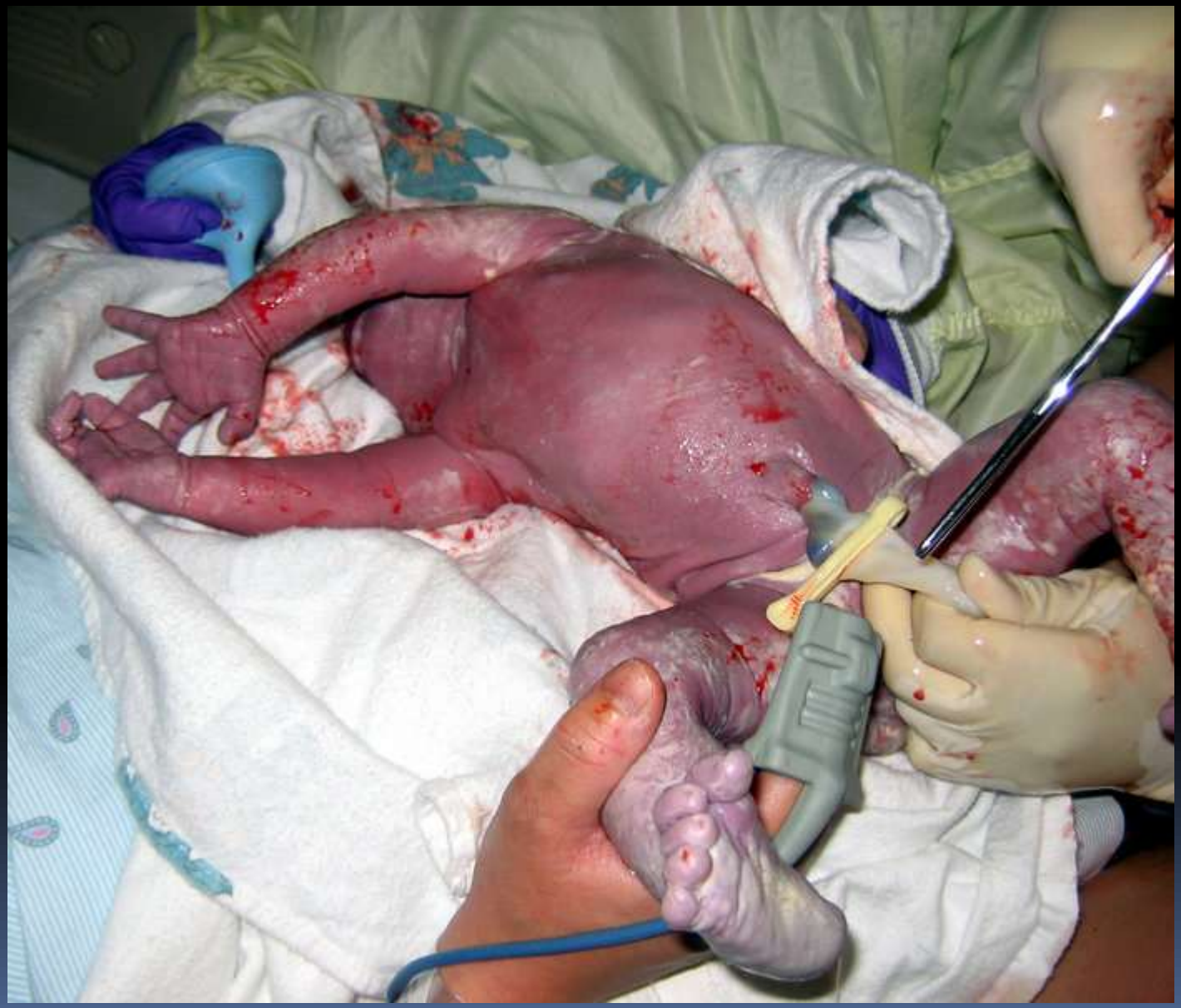
Sources of adult stem cells :

- Bone marrow
- Blood stream
- Umbilical cord blood
- Dental pulp of the tooth
- Cornea and retina of eye
- Skeletal muscle
- Liver
- Skin (epithelia)
- Gastrointestinal tract
- Pancreas
- Brain & spinal cord

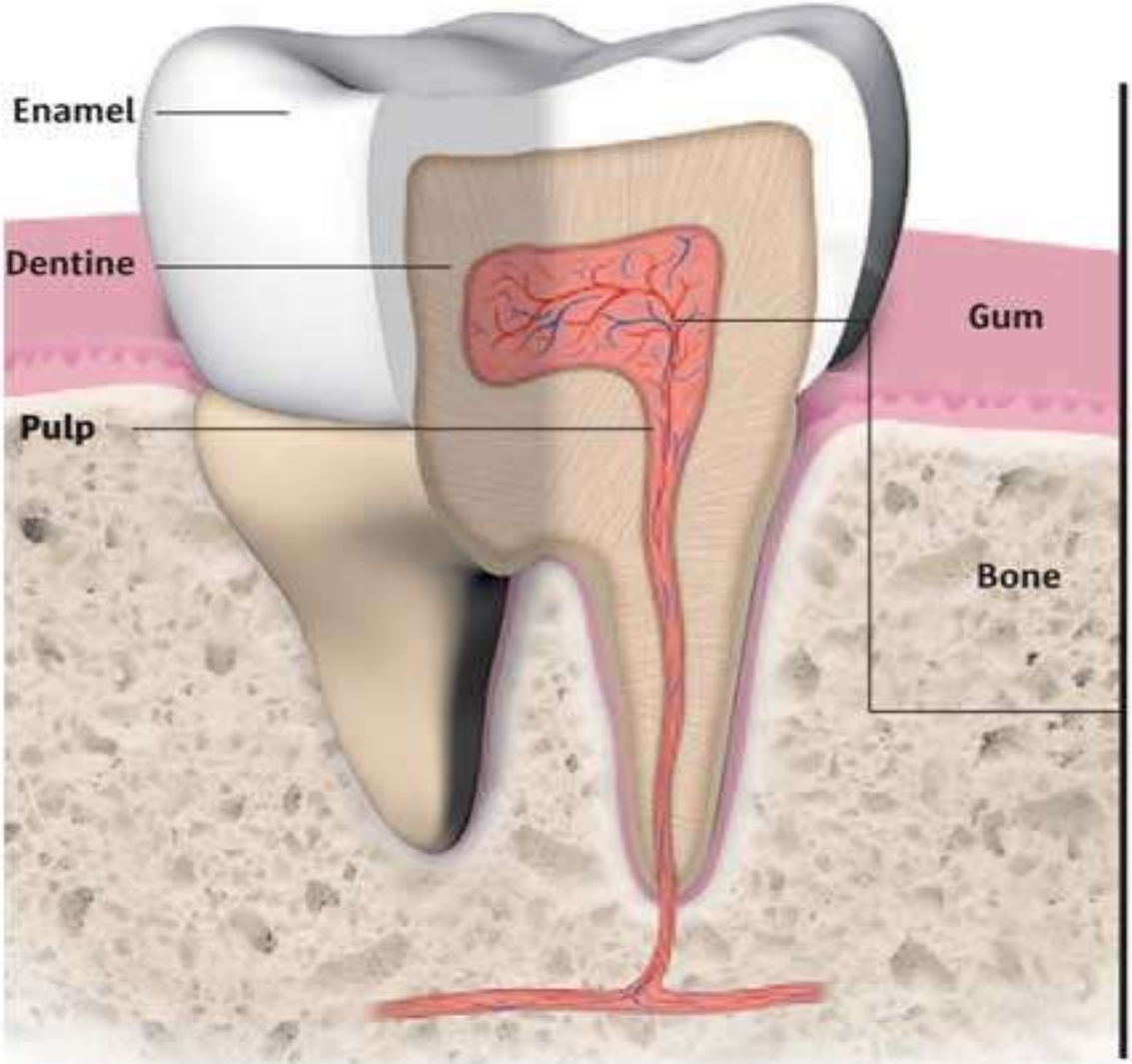
Bone marrow



umbilical cord blood



Dental Pulp



Stem cells are in areas next to nerve and blood vessels within the pulp of the tooth. Companies that bank stem cells say cells that can be regenerated from dental stem cells will someday include:

- Nerve and spinal cord
- Brain
- Heart
- Liver
- Bone
- Ligaments and cartilage
- Muscle
- Skin

Could a baby tooth one day save your child's life?

Adult stem cell plasticity

- **Plasticity**:

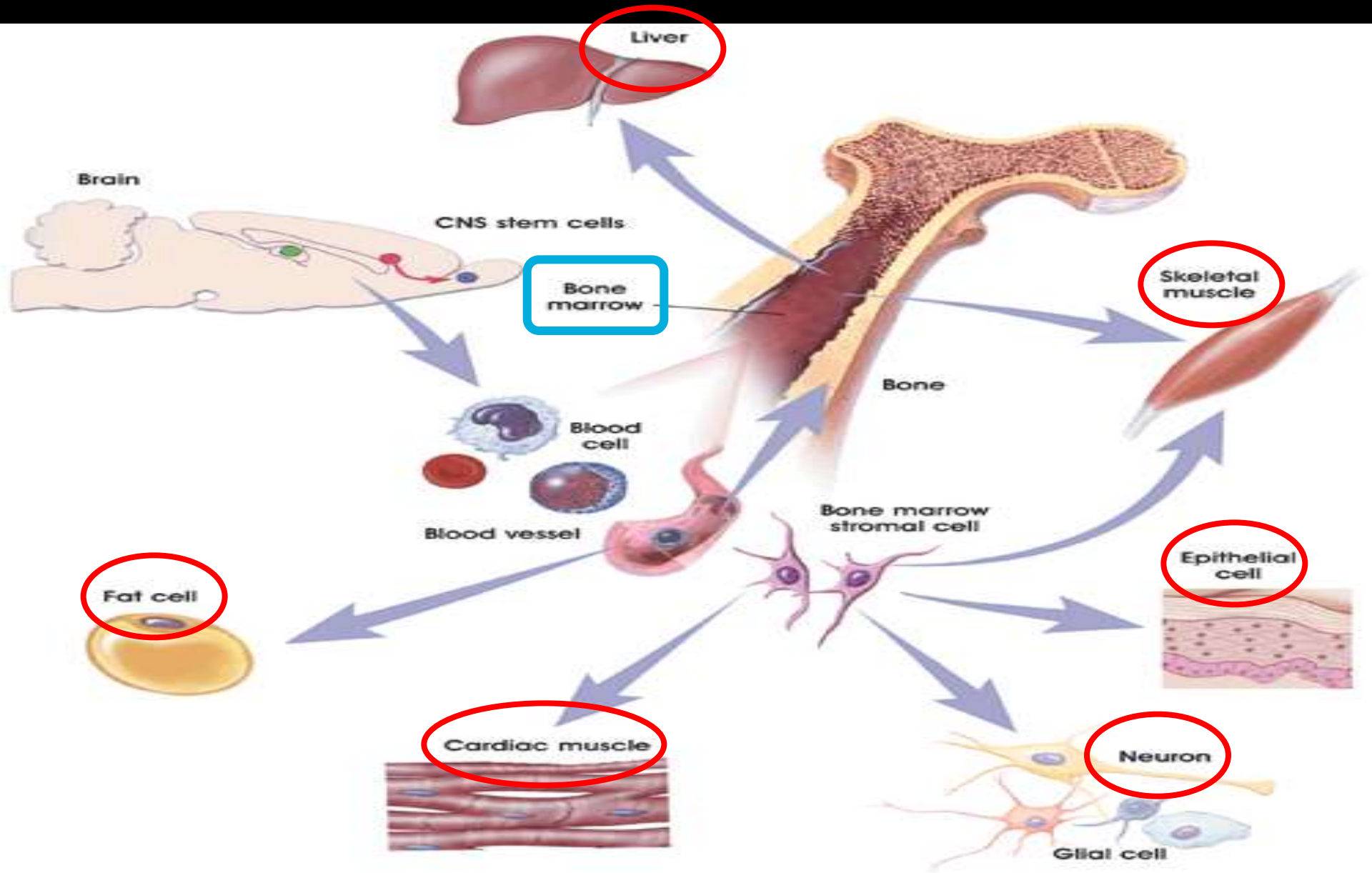
  stem cell from one adult tissue can generate the differentiated cell types of another tissue:

  "unorthodox differentiation" or "**transdifferentiation**"

- EX. Hematopoietic stem cell ⟶ Neurons

- Possible under specific conditions

Plasticity of adult stem cells



Advantages :

1. No immune attack
2. Available: eg HSC
3. Partly specialized: easier to control differentiation
4. Flexible: under the right conditions

Disadvantages :

1. Scarce (Rare): True for many Adult SCs
2. Unavailable: Some are difficult to isolate like Neural stem cells
3. Vanishing: Don’t live in culture as long as ES cells
4. Questionable quality: more brone to DNA abnormalities

Potential target disorders for Stem Cell Therapy

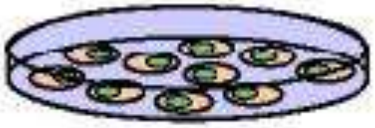
- Leukemia
- Heart damage
- Anemia
- Cornea damage
- Retinal damage
- Parkinson’s
- Alzhimer’s
- Diabetes
- Spinal Cord Injury
- Kidney Failure
- Skin grafts

Potential US Patient Populations for Stem Cell-Based Therapies.

Condition	No. of patients
Cardiovascular disease	58 million
Autoimmune diseases	30 million
Diabetes	16 million
Osteoporosis	10 million
Cancers	8.2 million
Alzheimer's disease	5.5 million
Parkinson's disease	5.5 million
Burns (severe)	0.3 million
Spinal-cord injuries	0.25 million
Birth defects	0.15 million/year

The Promise of Stem Cell Research

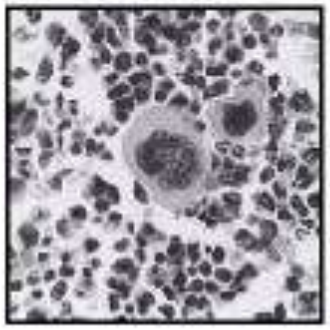
Drug Development and Toxicity Tests



Experiments to Study Development and Gene Control

Cultured Pluripotent Stem Cells

Tissues/Cells for Therapy



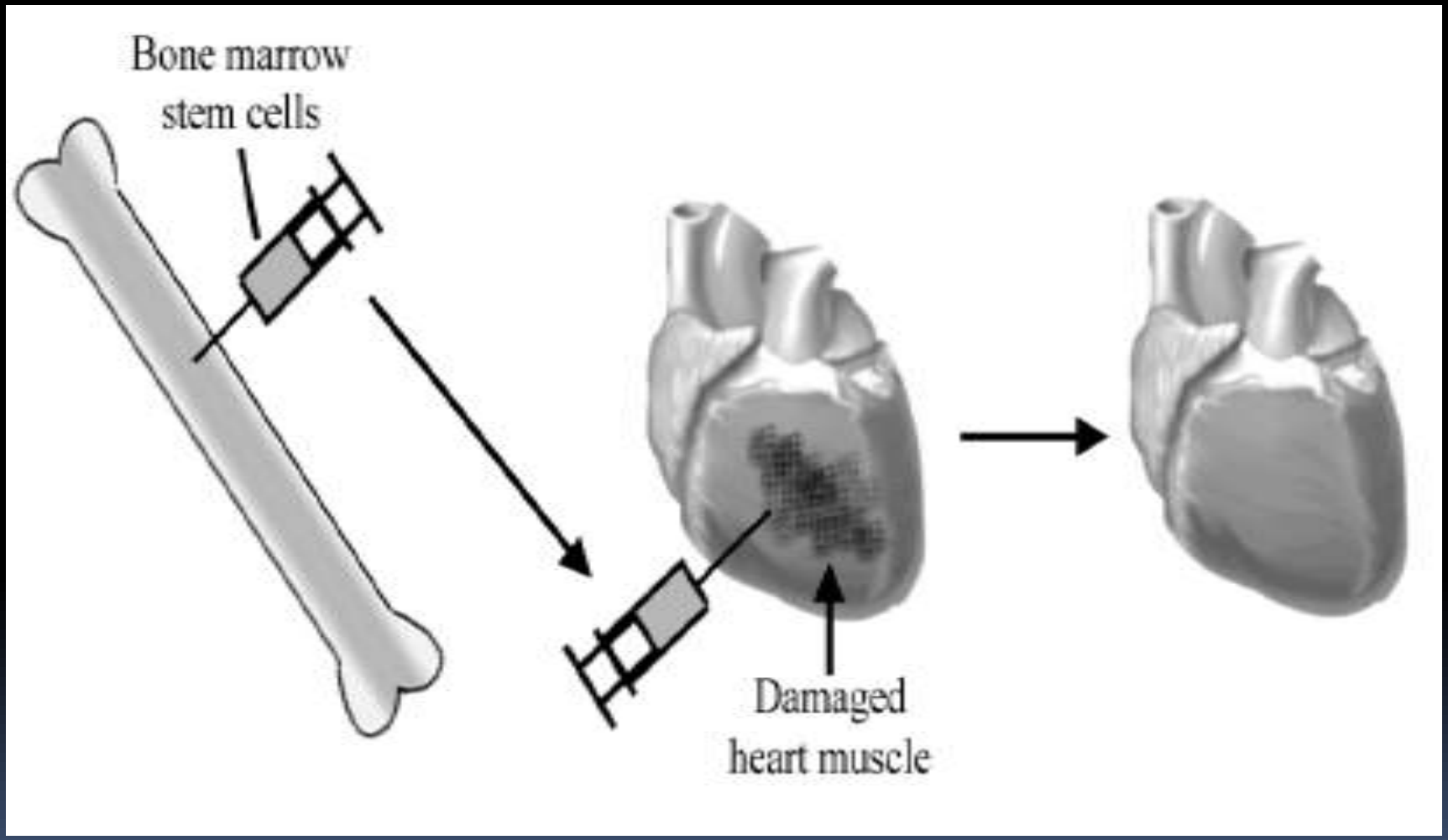
Bone Marrow

Nerve Cells

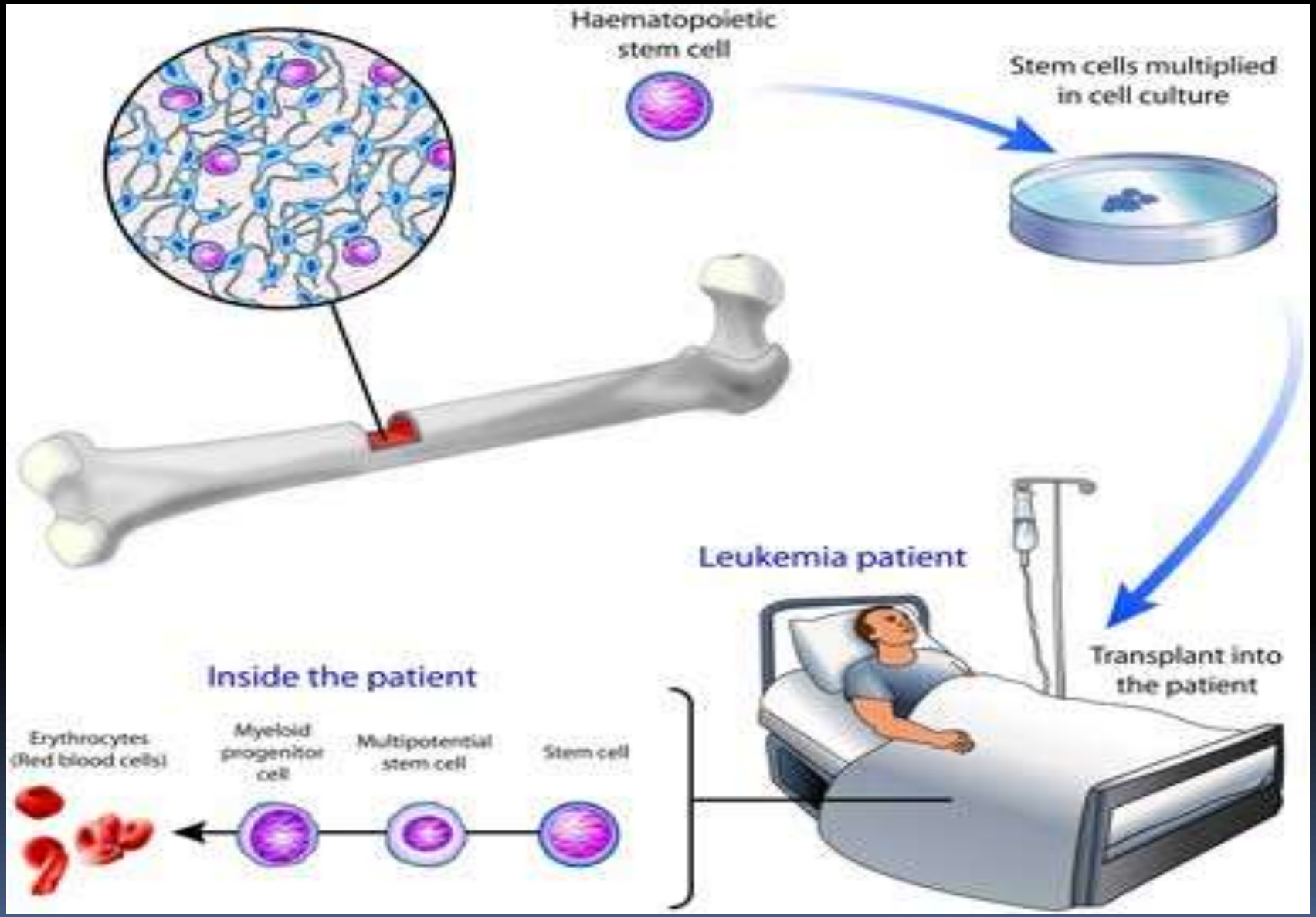
Heart Muscle Cells

Pancreatic Islet Cells

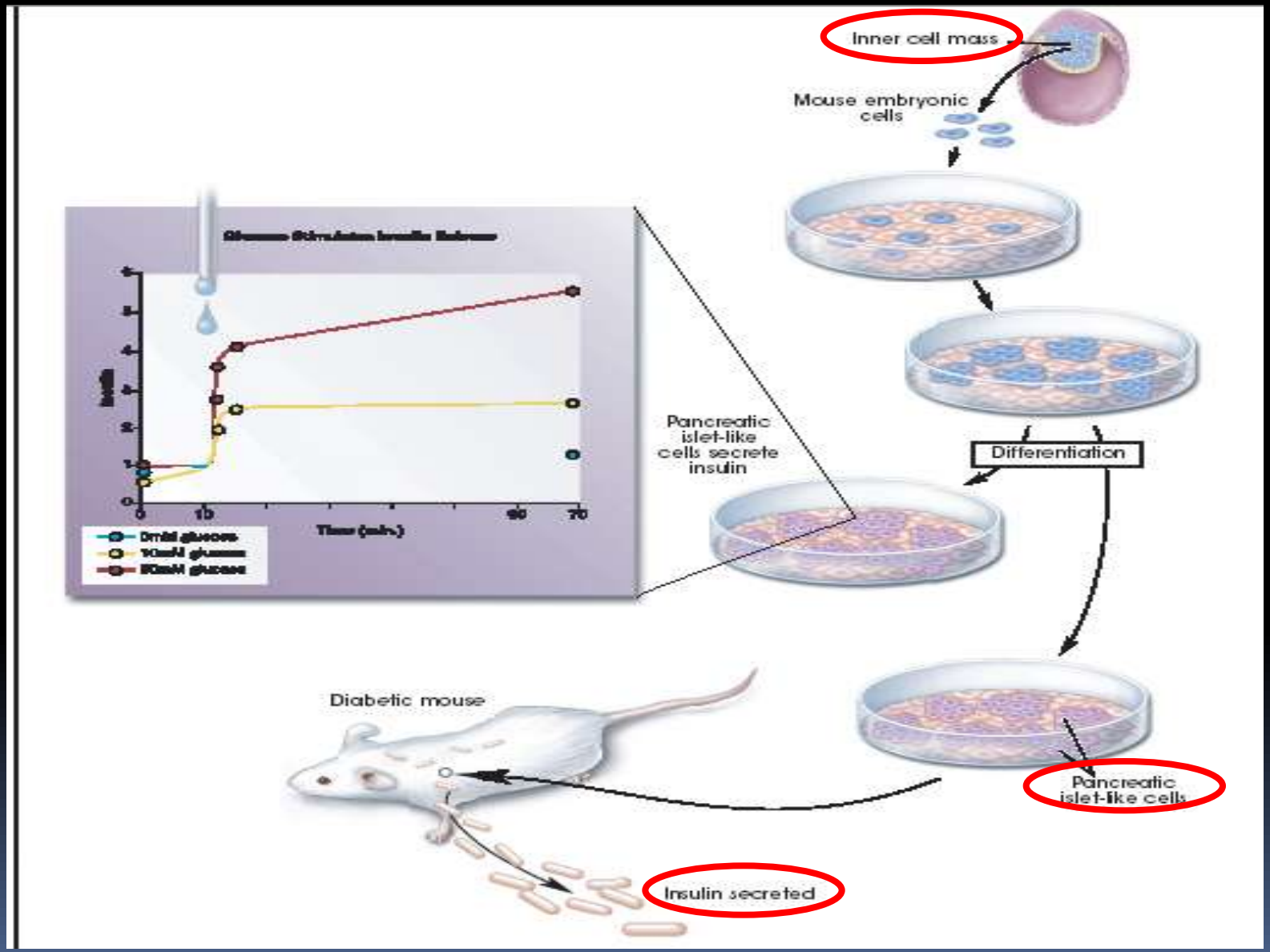
Heart damage



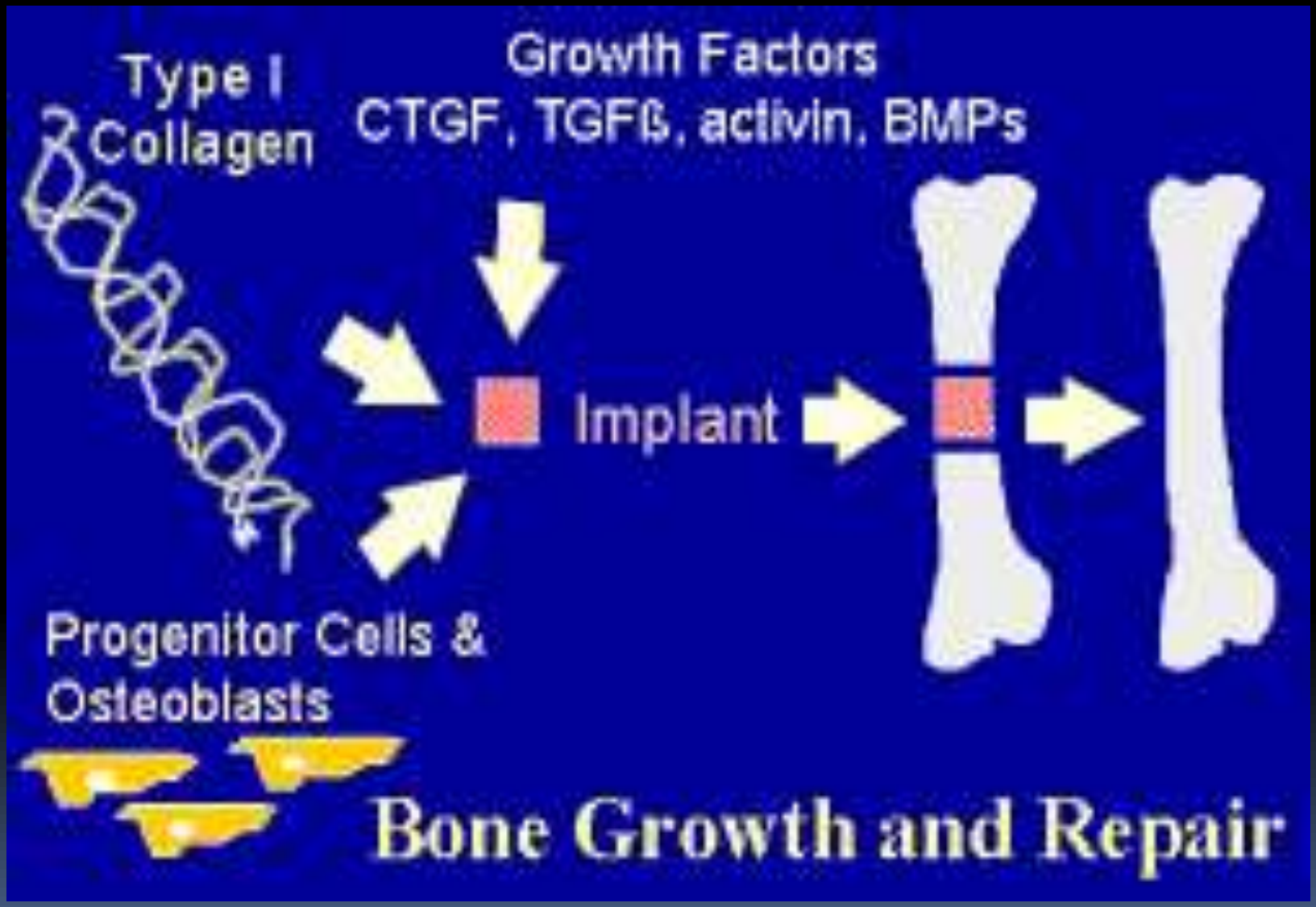
leukemia

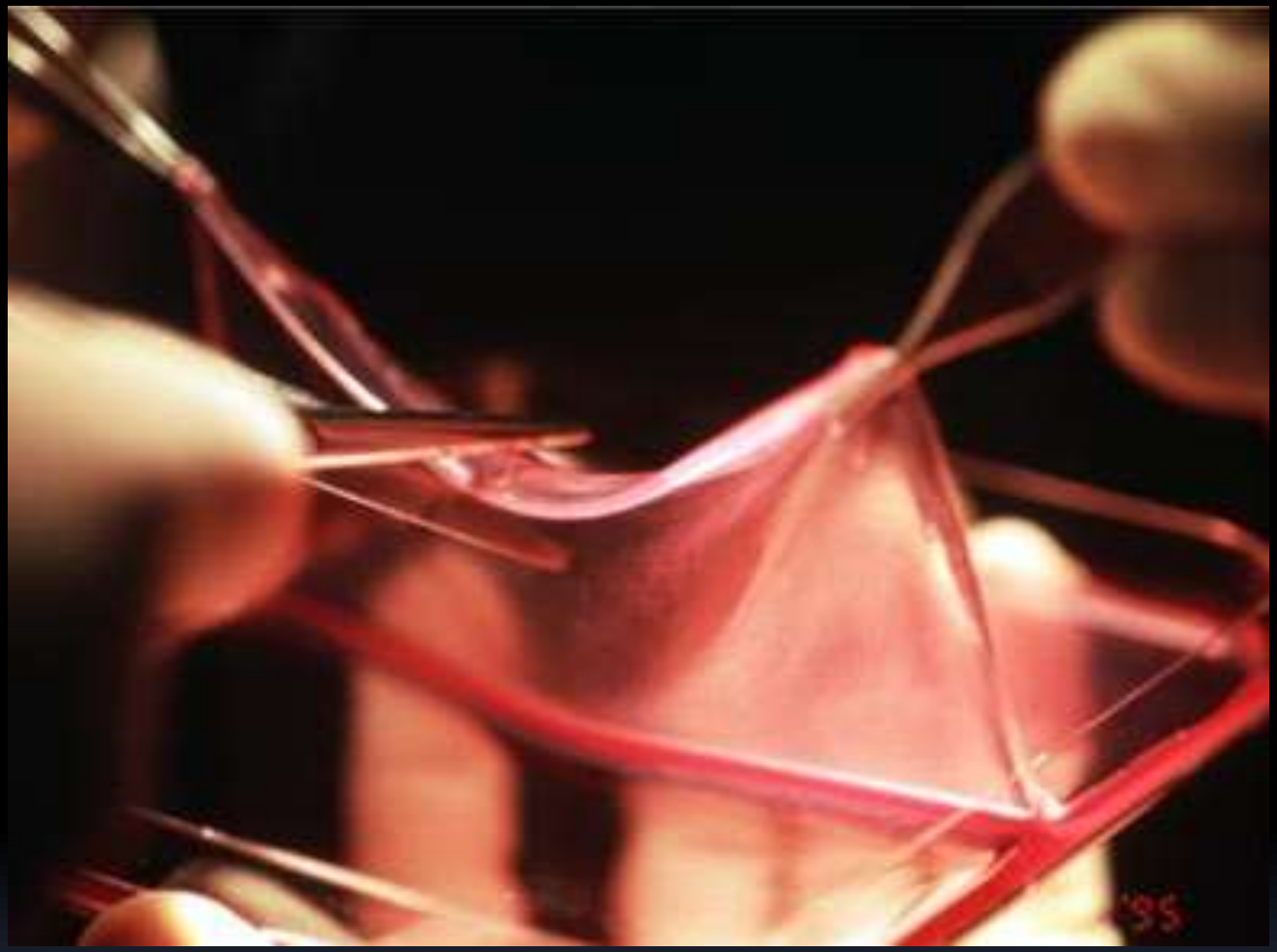

Haematopoietic stem cell
Stem cells multiplied in cell culture
Leukemia patient
Transplant into the patient
Inside the patient
Erythrocytes (Red blood cells)
Myeloid progenitor cell
Multipotential stem cell
Stem cell

Diabetes


Inner cell mass
Mouse embryonic cells
Differentiation
Pancreatic islet-like cells secrete insulin
Diabetic mouse
Pancreatic islet-like cells
Insulin secreted

Bone Repair

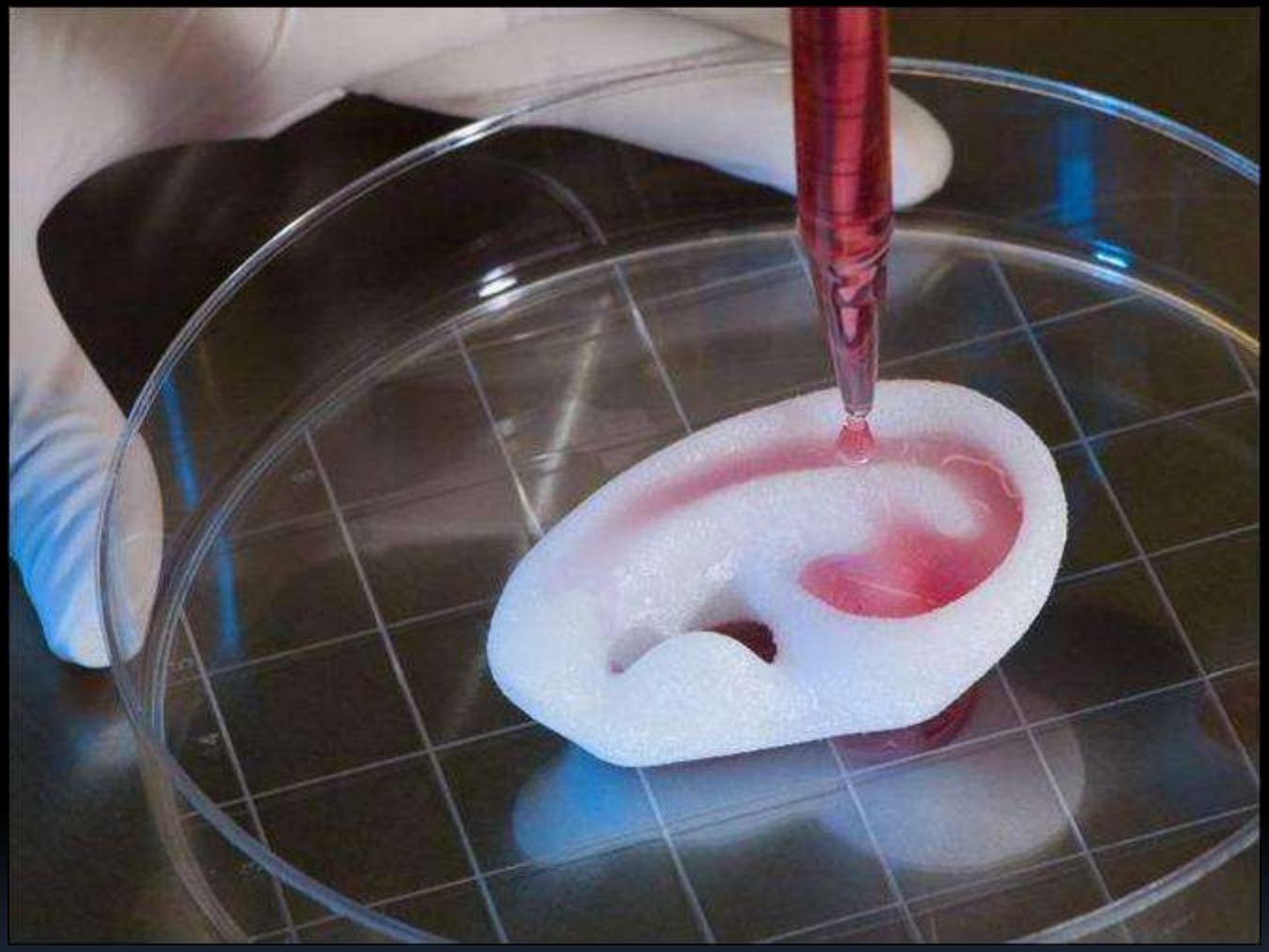

Type I
Collagen
Growth Factors
CTGF, TGFß, activin, BMPs
Implant
Progenitor Cells &
Osteoblasts
Bone Growth and Repair



Skin graft grown from stem cells

Cornea

trachea from stem cells



A grown ear seeded with cartilage cells

Retrodifferentiation = Re-programming = iPS

- Producing stem cells from differentiated cells !!!
- Pluripotent Embryonic like stem cells are produced
- Reversal of normal process
- Does Not require human embryos
- No donor…..No rejection
- Less expensive
- No Ethical issues

Induced pluripotent stem cell (iPS):

- Pluripotent !!

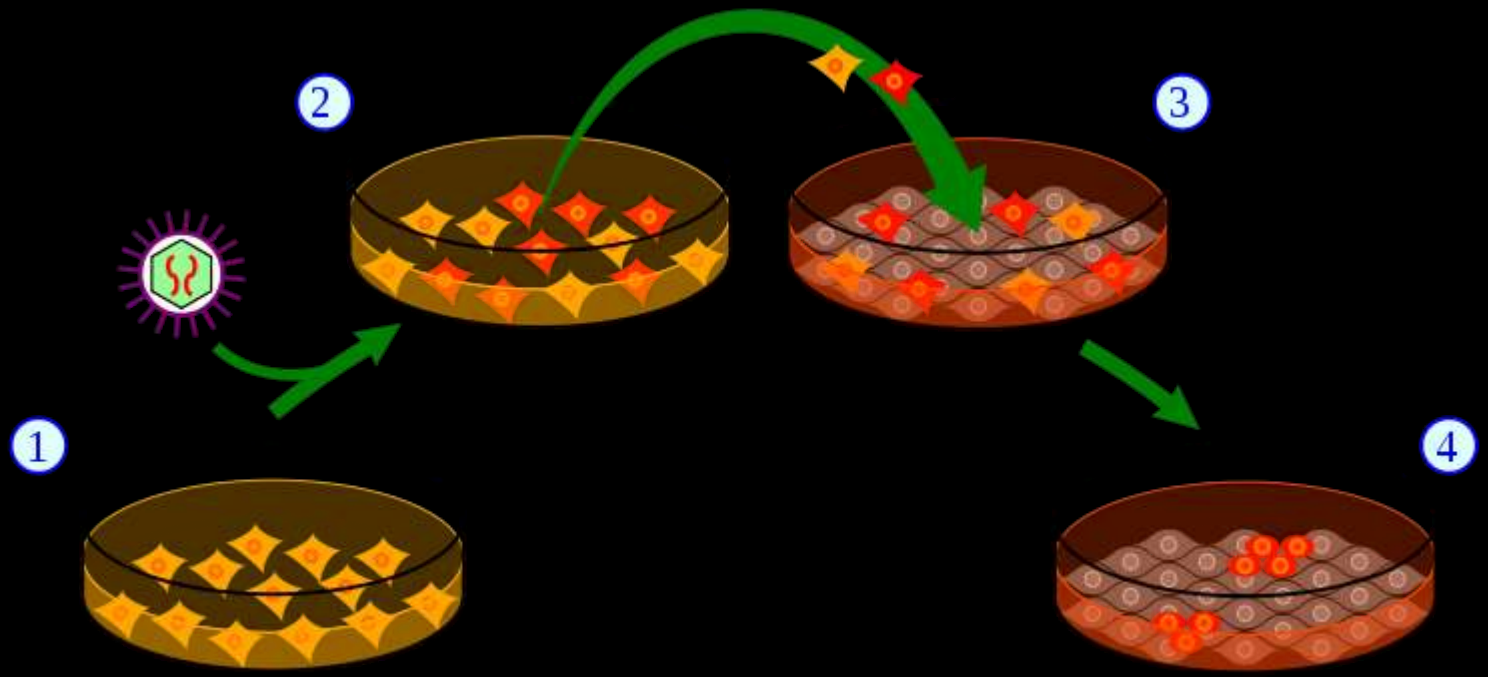
- Derived from adult somatic cells by inducing expression of certain Stemness genes: (usually by viral vectors: risk !!!)
    - eg: Master transcriptional regulators:
        - Oct-4
        - Sox2
        - Nonog
    - other genes: c-Myc (oncogene: cancer risk !!!!)

Cont: iPS

Believed to be identical to embryonic stem (ES) cells in many respects:

- expression of certain stem cell genes and proteins,
- chromatin methylation patterns
- doubling time
- embryoid body formation
- teratoma formation
- viable chimera formation
- potency and differentiability

Generation of induced pluripotent stem (iPS) cells



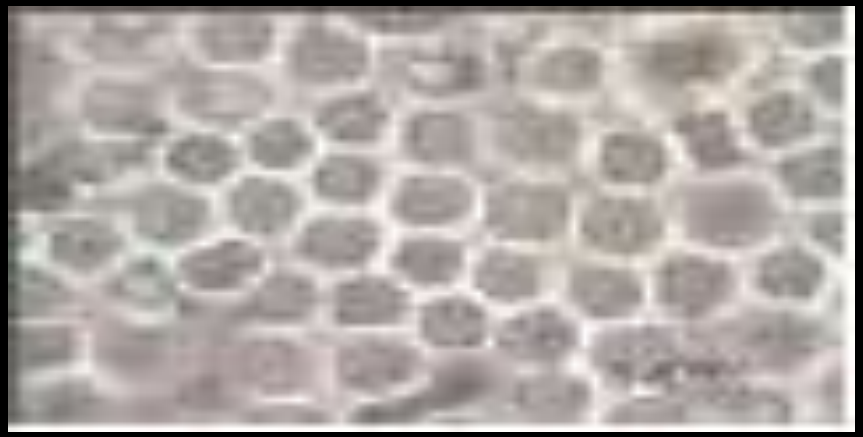
(1) Isolate and culture donor cells.

(2) Transfect stemness genes into cells by viral vectors. Red cells express those genes

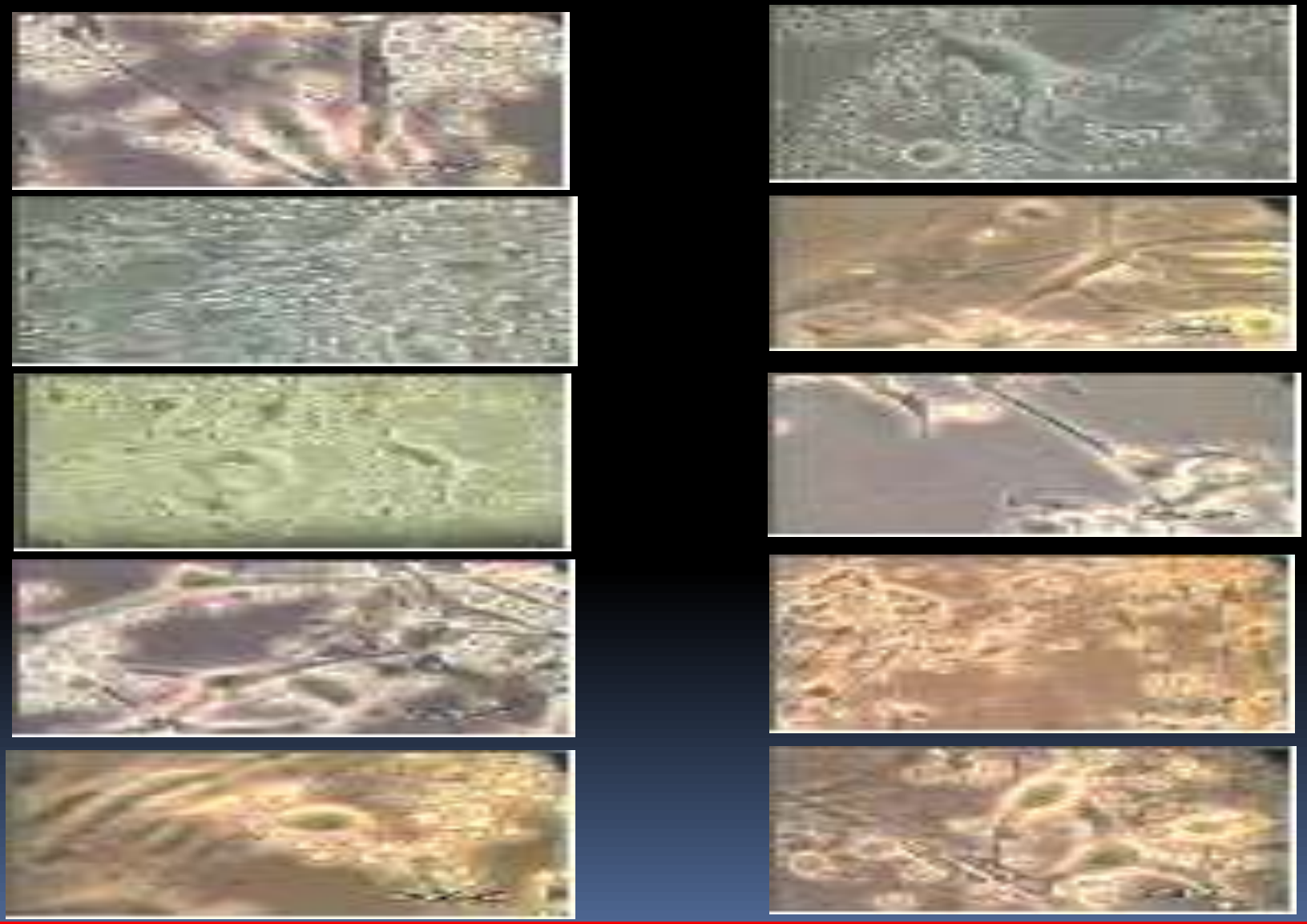
(3) Harvest and culture the cells according to ES cell culture, on feeder cells (lightgray)

(4) A subset of the transfected cells become iPS cells and generate ES-like colonies

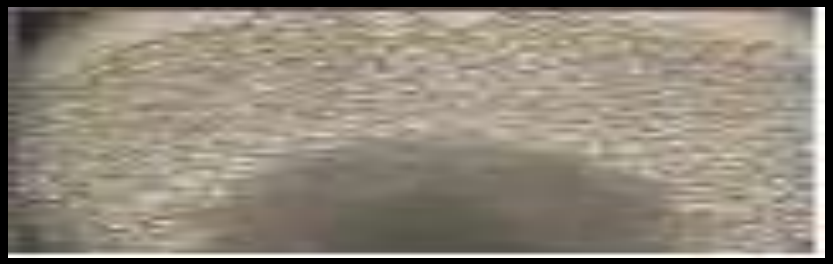
Haematopoietic Stem Cells Produced by Retrodifferentiation



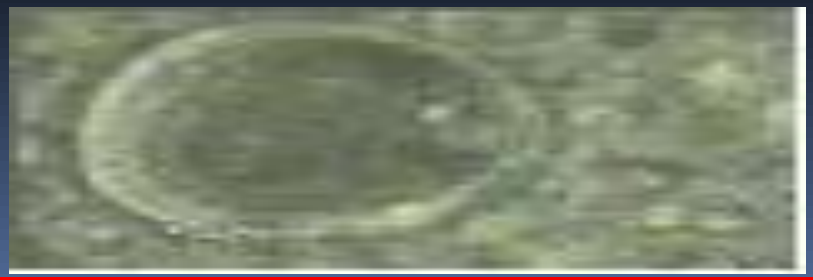
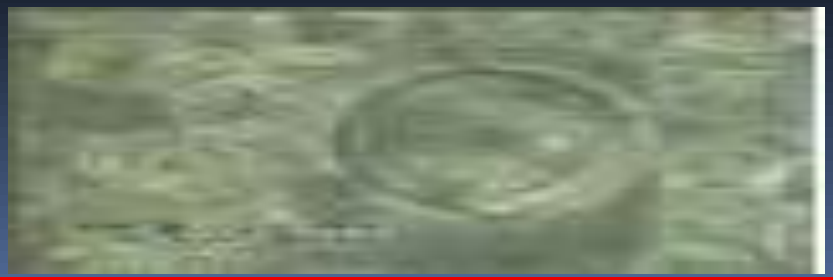
Neurogenesis of Retrodifferentiated Pluripotent Neuronal Stem Cells derived from Adult Leukocytes



Cartilage and Fat Cells Production by Retrodifferentiated Mesenchymal Stem Cells derived from Adult Leucocytes after Treatment



Ethical Concerns

- **Embryos**
  - Embryo can develop into a person
  - Unused embryos are disposed of
- **Fetal tissue**
  - Fetus is already dead
  - Closely associated with abortion (Could make abortion more acceptable)
- **Adult stem cells**
  - Alternative source but it is more limited

Global overview on embryonic stem cells

Europe:

- Most countries: OK !! (including UK)

USA:

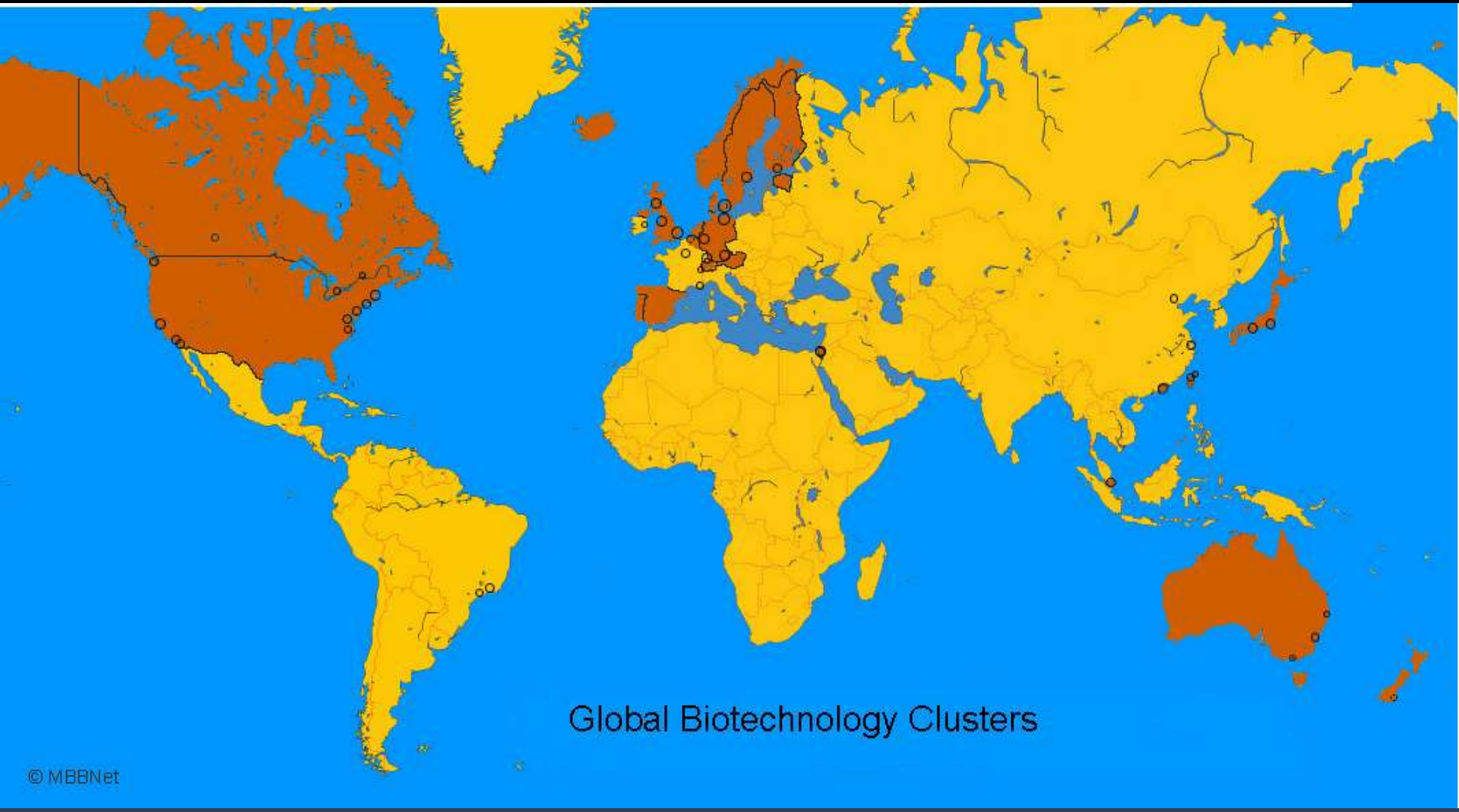
- 2001: Bush banned federal funding
- 2009: Obama lifted the restrictions

Asia:

- Least restrictions


IT'S UNETHICAL TO SPEND TAXPAYER DOLLARS TO DESTROY HUMAN EMBRYOS.
HUMAN BEINGS- OK.
STEM CELL RESEARCH VETO
IRAQ
U.S.
CIVILIANS

Stem Cell Research: Global Competition

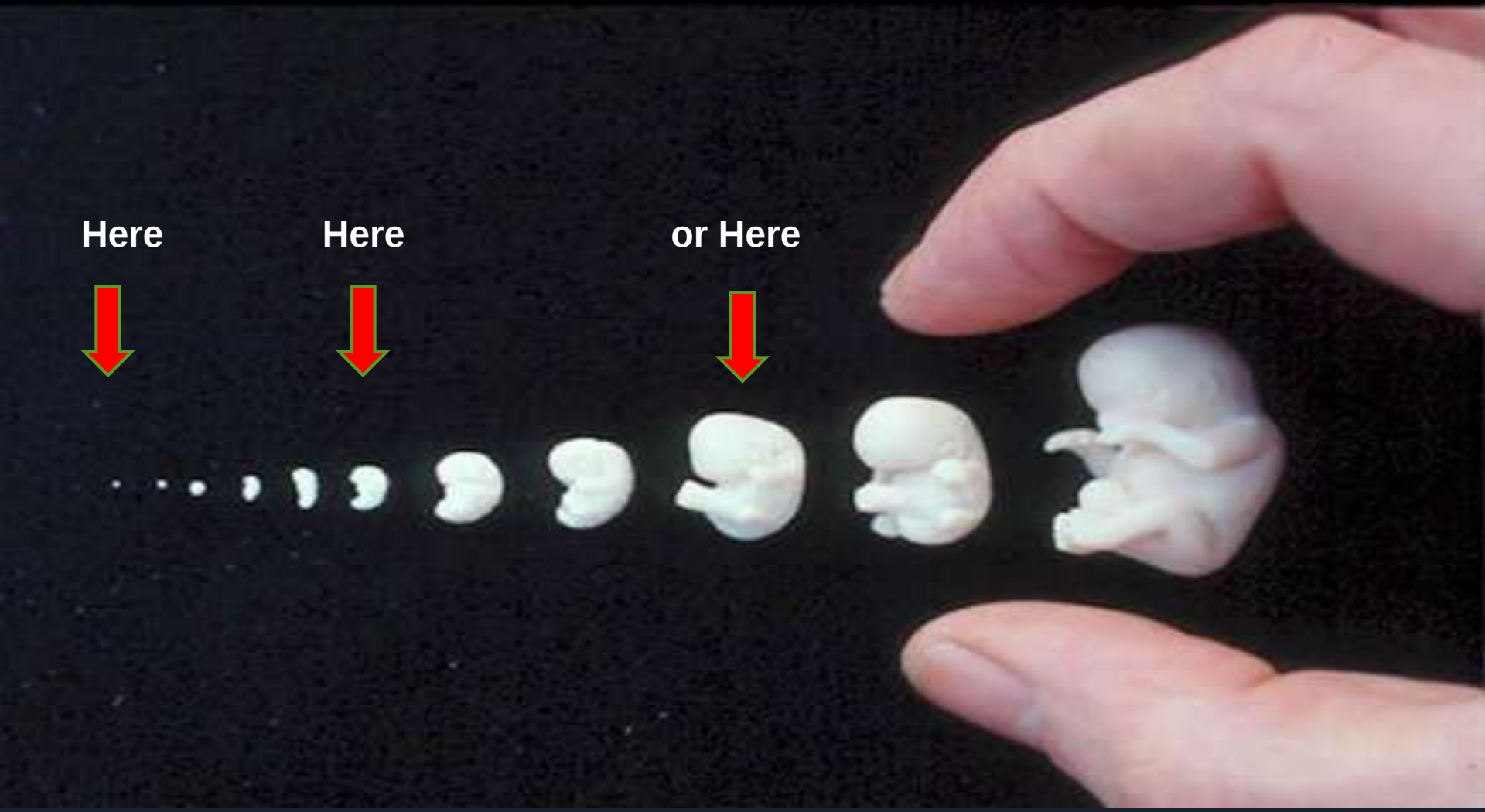


Countries in brown: Advanced stem cell research

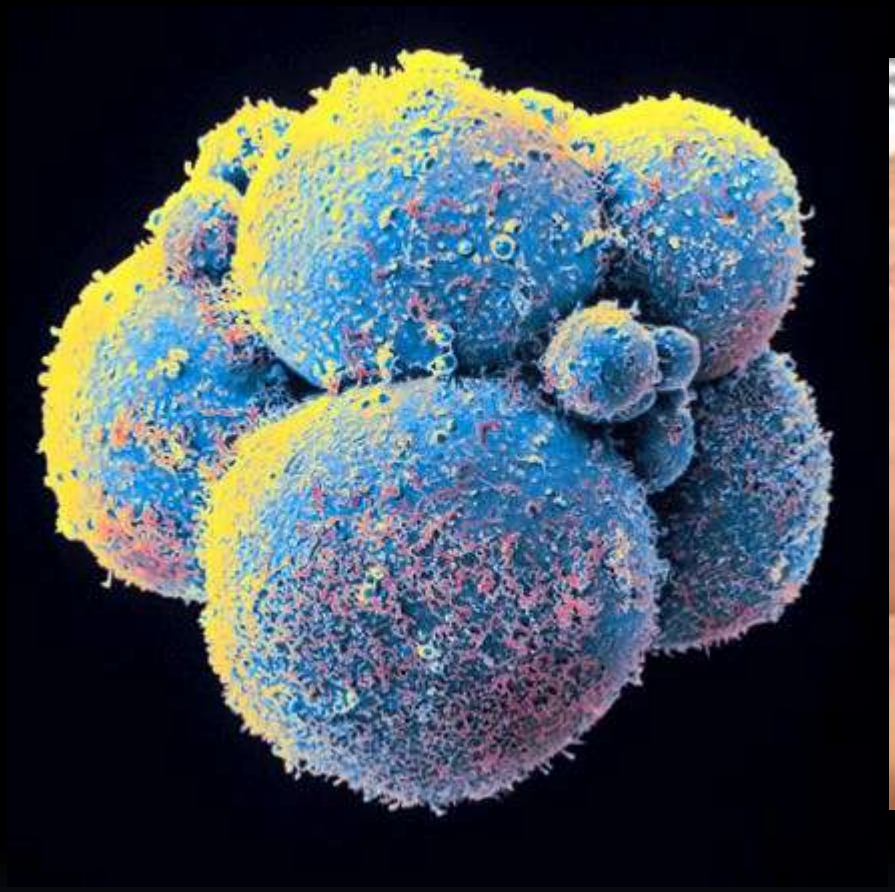
Stem Cell Research: Regulations

Countries in brown:

- Representing > 3 billion people,
- Permissive / flexible policy on embryonic stem cell research
- All have banned human reproductive cloning.



When is it OK….when is it NOT



Group of cells **or** **Human life**


I DIED WAITING FOR EMBRYONIC STEM CELL RESEARCH TO FIND A CURE. WHAT ABOUT YOU?
I WAS THE EMBRYO

THANK YOU