Previous Class

- Gene Therapy
  - Ex vivo
  - In vivo
  - Vectors used
  - Other methods: naked DNA, liposomes, antisense RNA and RNAi technologies
  - SCID patients (toxicity due to ATP accumulation in T-cells)
  - CFTR (Cl- ion accumulation in tracheal cells)
- Regenerative Medicine
  - Fetal tissue grafts (Bridge implants)
  - Organ transplantation : Autografting
    - Rejection of donated organs
    - HLA marker from MHC complex

Xenotransplantation

- Transplanting organs from one species into another
- May someday become an alternative to human-to-human transplantation
  - 1984 baboon heart transplanted into a 12-year-old human girl
    - Girl died after 3 weeks as a result of organ rejection
  - Pigs may be good choice for organ transplantation
    - They are plentiful
    - Easy to breed
    - Inexpensive
    - Many pig organs are similar in function and size to human organs
    - Limitation: viruses may be transmitted from pigs to humans, causing rejection of transplanted organ and other health problems

- Molecular techniques and transplantation techniques are combined to produce **cloned pigs** that may help overcome rejection and viral disease transmission problems
- Normal pigs contain a gene ***GGTA1*** (b-1,3-galactosyl-transferase) which produces a **sugar on surface of pig tissues**, which when transplanted into human, would be recognized as foreign antigen, leading to antibody production and **rejection of the organ**
- In cloned pig, the gene ***GGTA1*** **is knocked out** using nuclear transfer cloning technique

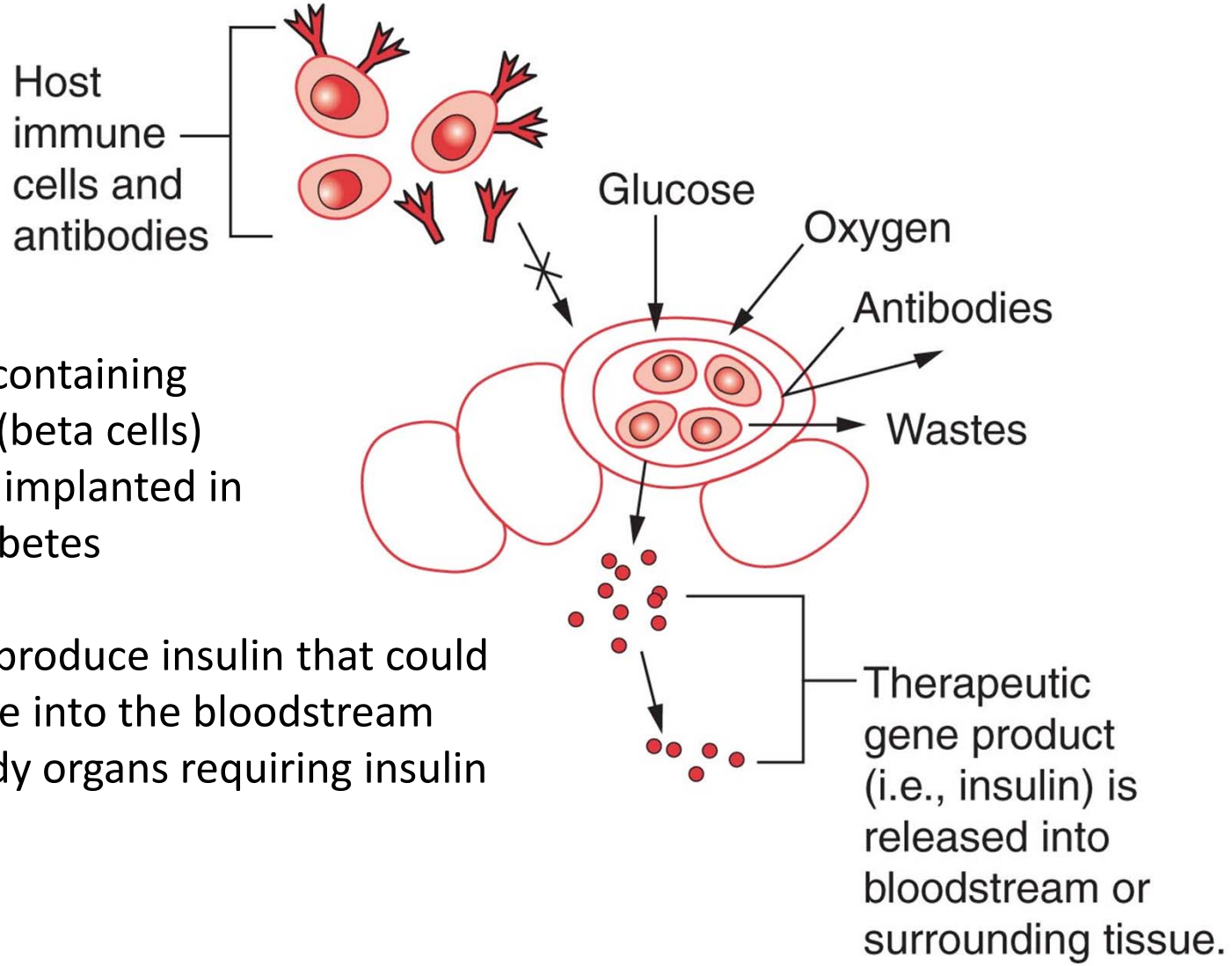
Cellular therapeutics

- Cells are used to replace defective tissues or to deliver important biological molecules
- Living cells or genetically engineered cells to produce therapeutic molecules or recombinant proteins are encapsulated into tiny plastic beads or tubes are called **biocapsules** or microcapsules

Biocapsules

Have tiny holes in their walls so nutrients can get in and therapeutic molecules can come out

Are protected by the attack of recipient's immune system



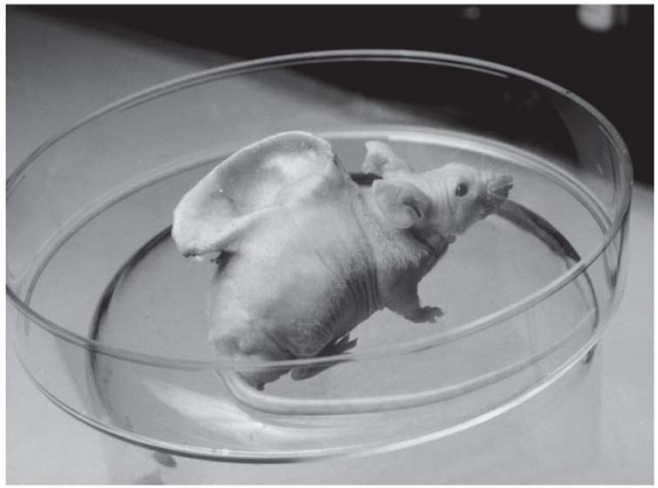
For example, capsules containing insulin-producing cells (beta cells) from the pancreas, are implanted in patients with type I diabetes

These biocapsules will produce insulin that could travel out of the capsule into the bloodstream of the patient to all body organs requiring insulin

Tissue Engineering

- May provide tissues and organs that can be used to replace damaged or diseased tissues
- Process
  - Design a framework or scaffold
  - Seed the scaffold with human cells
  - Bathe in nutrient-rich media
  - Cells will build layers and assume the shape of the scaffold

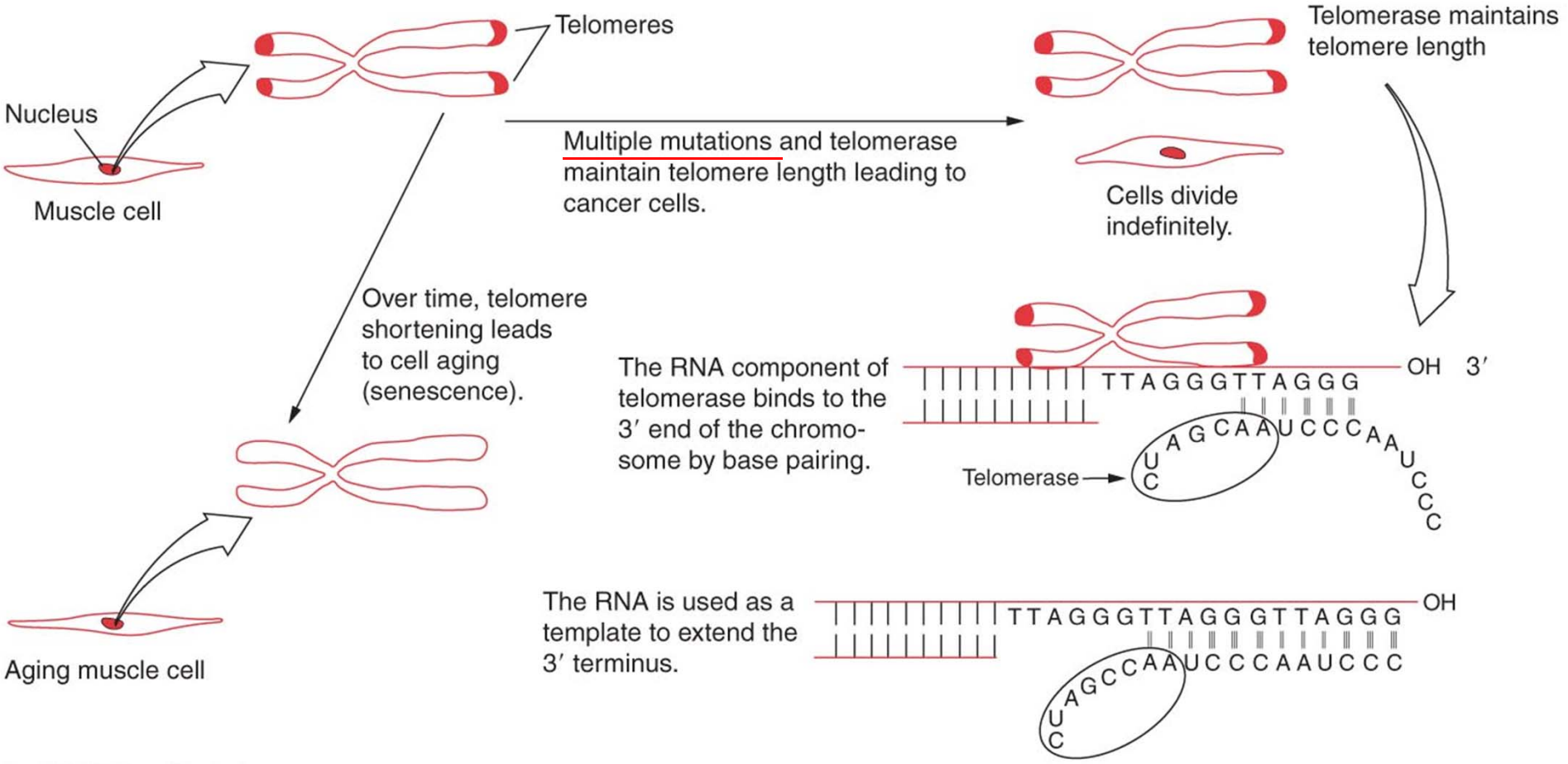
- Tissue Engineering
  - Sheets of skin grafts
  - 1990s Dr. Charles Vacanti revealed a mouse with an engineered ear growing on its back
    - Seeded with cells from a cow
    - Just the outer ear without the inner ear structures that actually detect sound
  - Human bladders, rudimentary kidney



The Telomere Story

- **Telomeres** are DNA sequences found on the end of chromosomes. Usually 8 – 12K units of repeating sequence 5'-TTAGGG-3'
- Most human cells can divide a maximum of 50 – 90 times before they age – a process called **senescence** – which leads to cell death
- Life span of cell is affected by telomeres
  - Each time a cell divides, telomeres shorten slightly
  - This occurs because of a basic flaw that prevents DNA polymerase from completely copying the ends of both strands of a DNA molecule
  - Eventually loss of the repeat sequences produces a critical loss of DNA so cells can no longer divide

– **Telomerase** is an enzyme that repairs telomere length at the ends of chromosomes after each round of cell division
  - Not active in normal cells
  - But is active in 90% of human cancer cells, making them divide indefinitely



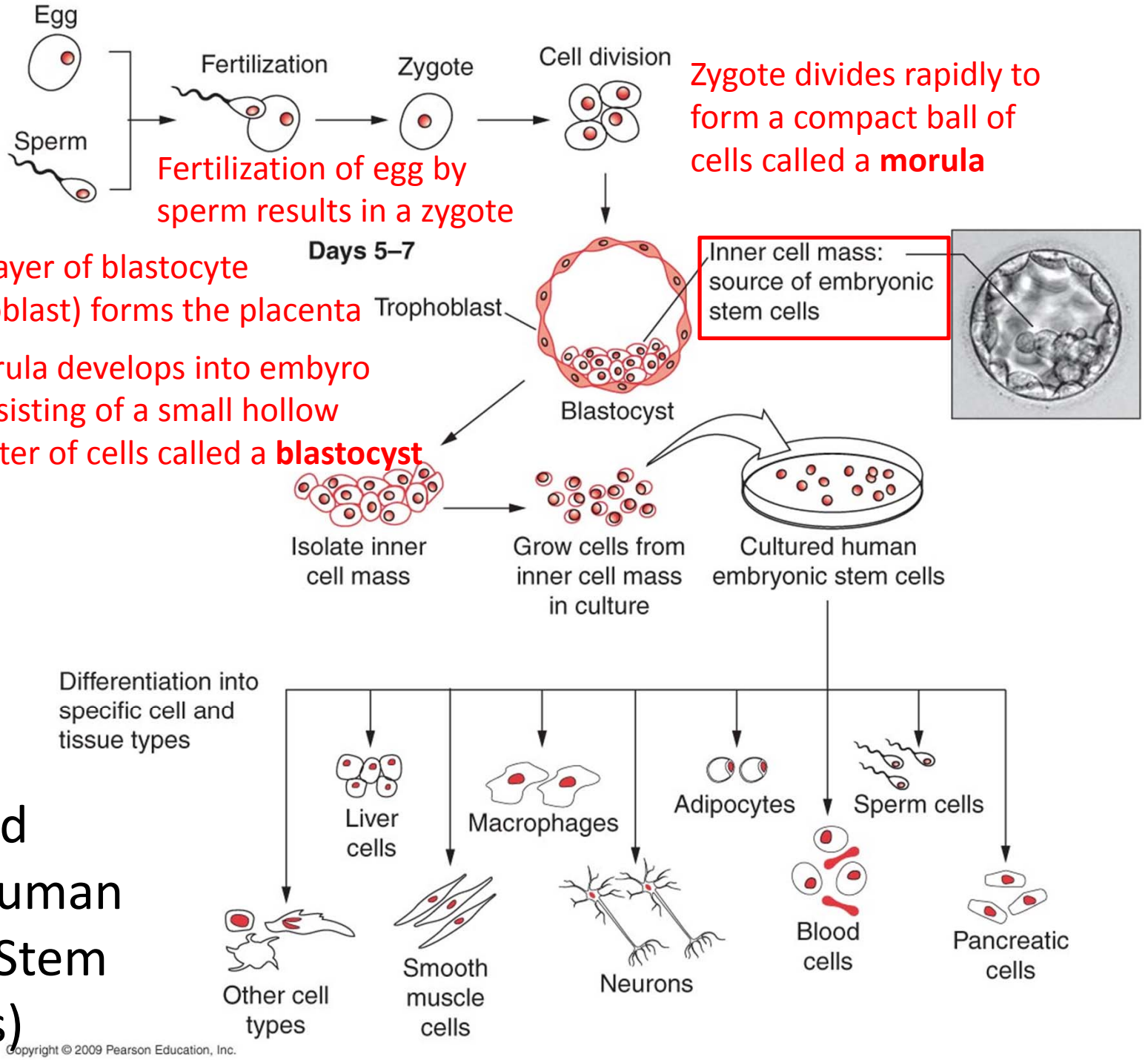
- Scientists are investigating how introducing telomerase genes into cultured human cells can allow them to produce normal human cells that display immortality
- Useful for treating age-related disorders, arthritis, neurodegenerative diseases.
- Provide skin-cells for healing bedsores, ulcers, and treating late-onset blindness, and muscular dystrophy

Stem Cells

What are Stem Cells?

- Stem cells are typically those cells which can undergo **self-renewal** and **differentiation**
  - **Self-renewal:** stem-cells grow and divide (proliferate) indefinitely by mitosis to create populations of identical stem cells.
  - **Differentiation:** Stem-cells can differentiate to form all of the more than 200 different cell types that make up human body, so they are called **pluripotent**. This is a complex process and requires many genes and chemical signals.

Isolating and Culturing Human Embryonic Stem Cells (hESCs)



- hESCs can self-renew indefinitely to produce more stem cells
  - hESCs avoid senescence and show no aging
  - Express high levels of telomerase
  - Cultured hESCs, which can be maintained and grown successively, are called **cell lines**.
  - Stem cell lines are stimulated with hormones and growth factors to differentiate into different types of cells.
  - Can be used to create tissues for regenerative medicine
  - ESCs from human, mice, rats, primates, etc have been used to form myriad of cells including skin, brain, cartilage, spermatozoa, osteoblasts, liver, insulin-secreting pancreatic beta cells, muscle cells, cardiac muscle cells etc
- Source of hESCs
  - Embryos left over from IVF (**in vitro fertilization**)
  - Embryos created by IVF from sperm and egg cells donated for the purpose of providing embryos for research materials

Can **adult stem cells** do everything embryonic stem cells can do?

- Cells that reside in mature adult tissue and could be cultured and differentiated to produce other cell types – **adult-derived stem cells (ASCs)**
- Can differentiate into another different specialized cell type, but may not be as pluripotent as hESCs
- ASCs isolated from the heart, brain, intestine, hair, skin, pancreas, bone marrow, fat, mammary glands, teeth, muscle, and blood.
- Small in number and not yet discovered in all adult tissues

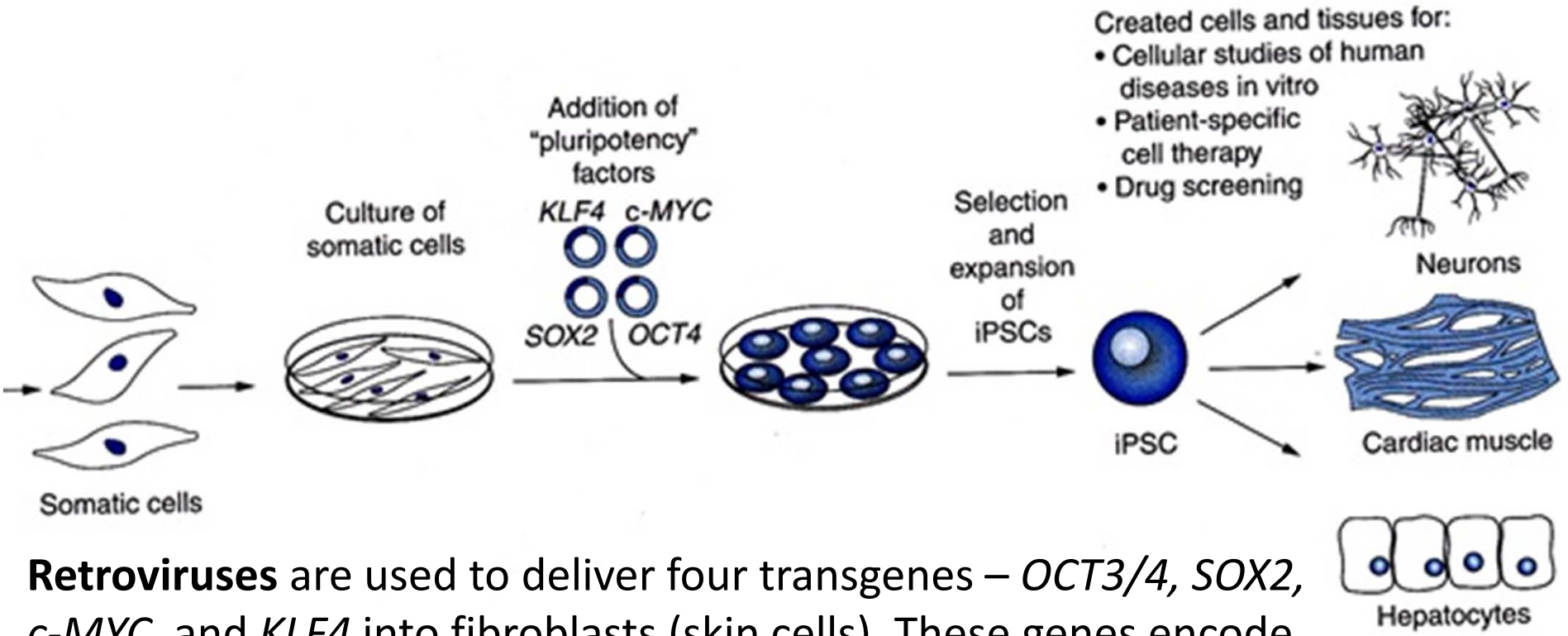
Can stem cells be generated without destroying an embryo?

- One approach involves creating stem cell lines from a single cell from the morula
- Amniotic fluid-derived stem (AFS) cells: Amniotic fluid is the protective fuild surrounding a developing fetus.
- Cancer stem cells (CSCs), tumor initiating cells: CSCs can self-renew and differentiate to form tissues from which they are derived

Creating stem cells by Nuclear Reprogramming of Somatic Cells

- Use genes involved in cell development to push a somatic cell back to an earlier stage of development and affect gene expression and thus to reprogram the somatic cell genetically to return to a pluripotent state characteristic of the stem cells from which it was derived.
- Earlier it was thought that once cells differentiated to become a specific, specialized cell types, they can not be differentiated again to form another cell type.
- But this is not so!

Induced Pluripotent Stem (iPS) cells



Retroviruses are used to deliver four transgenes – *OCT3/4, SOX2, c-MYC*, and *KLF4* into fibroblasts (skin cells). These genes encode transcription factors involved in cell development, so "reprograms" the fibroblast cells back to an earlier stage of differentiation. These reprogrammed cells are called **induced pluripotent stem cells (iPSCs)**.

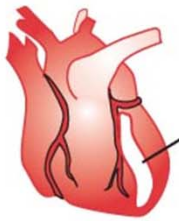
- iPSCs, like hESCs, can self-renew and are pluripotent.
- iPSCs are produced from human, mouse, rat, pig, and monkey cells.
- iPSCs have been successfully derived from human skin cells from patients.

Applications and limitations of iPSCs

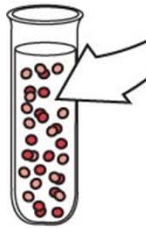
- iPSCs can be used for patient-specific cell therapies without the risk of immune rejection
- Disease specific stem cells can be created to
  - understand disease progression and processes
  - Drug screening tests
  - In vitro disease modeling
- Limitations
  - iPSCs are relatively inefficient to produce
  - Require constant feeding to maintain viable cell lines
  - Can be prone to forming tumors

Potential Applications of Stem Cells

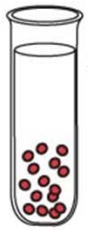
- Using stem cells to make white blood cells is becoming an effective way to treat leukemia
- Stem cells from umbilical cord blood used to treat sickle cell anemia and other blood deficiencies
- Stem cells from fat have been used to form bone tissue in the human skull
- Repair of heart cells
- Adult stem cells isolated from brain and used to make neurons in culture



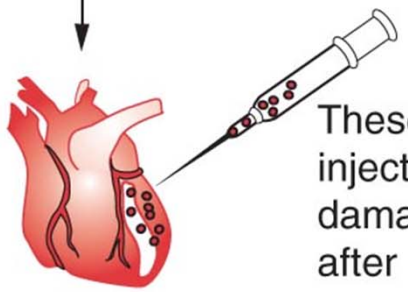
A heart attack was induced in a female mouse, causing damage (white area) to the left ventricle of the heart.



Bone marrow cells were taken from an adult, male mouse.



Stem cells were isolated from the bone marrow cells.



These stem cells were injected into the female's damaged heart 3 to 5 hours after its heart attack.

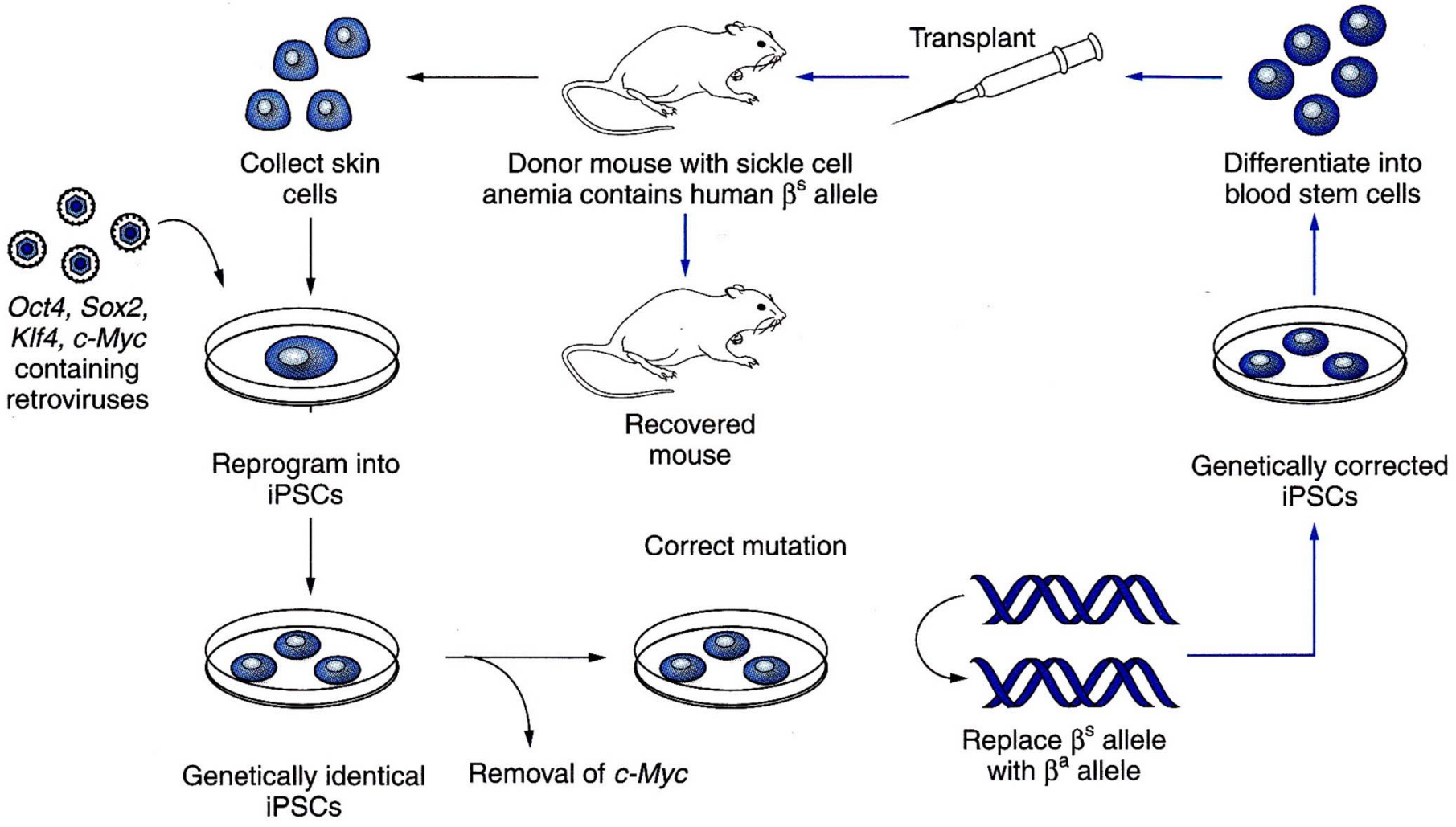
A week or two afterward, the injected cells helped regenerate part of the damaged heart. The presence of a Y chromosome identifies the male donor cells.

Repairing a Damaged Heart with Adult Bone Marrow Stem Cells.

Mouse adult bone marrow stem cells can be used to repair areas of the mouse heart damaged by a heart attack. These stems cells can develop into cardiac muscle cells, form electrical connections with healthy muscle cells, and improve heart function by over 35%.

hESCs have been transplanted in similar way

Treating Sickle cell Anemia with iPSCs



Issues related to use of Stem Cells

- How to control the differentiation of pluripotent stems cells into desired tissue of interest?
- When injected, the spread of stem cells to other places in the body can not be controlled
- Injected hESCs have formed tumors, including *teratomas*, which contain mixtures of differentiated tissues such as teeth, bone, and hair, all in one tumor.
- Differentiating stem cells suffer from chromosomal abnormalities (trisomy 21, trisomy 17, etc)

Unanswered Questions in Stem Cells Research

- Why do stem cells self-renew and maintain an undifferentiated state?
- What factors trigger the division of stem cells?
- What are the growth signals (chemical, genetic, environmental) that influence the differentiation of stem cells?
- What factors affect the integration of new tissues and cells into existing organs?
- Can nuclear reprogramming of somatic cells or other approaches that do not require embryo become reliable techniques for producing pluripotent stem cells with properties of hESCs?
- Which diseases can be most effectively treated by stem cell technologies?
- What strategies will be most effective for delivering stem cell treatments?