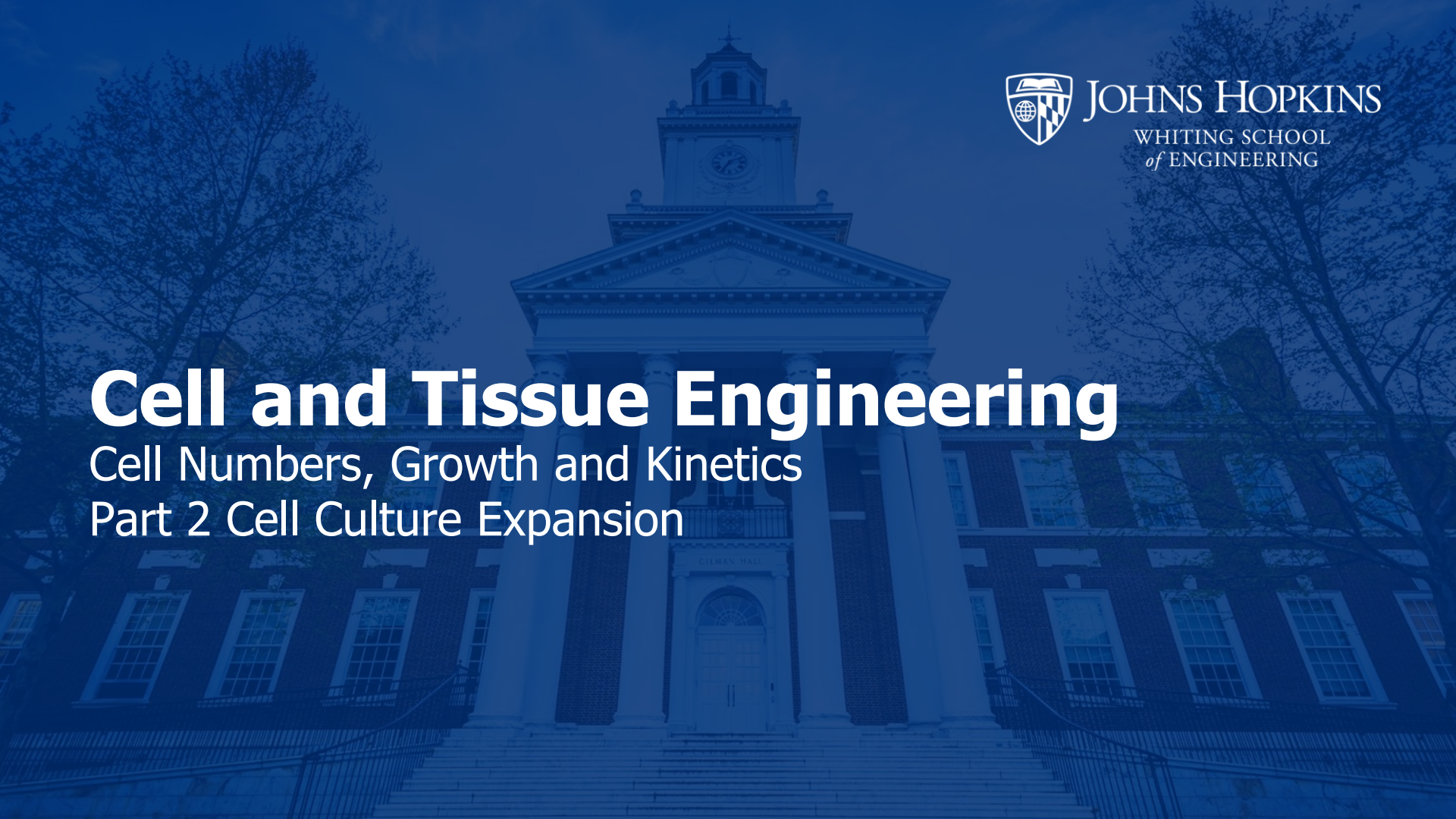
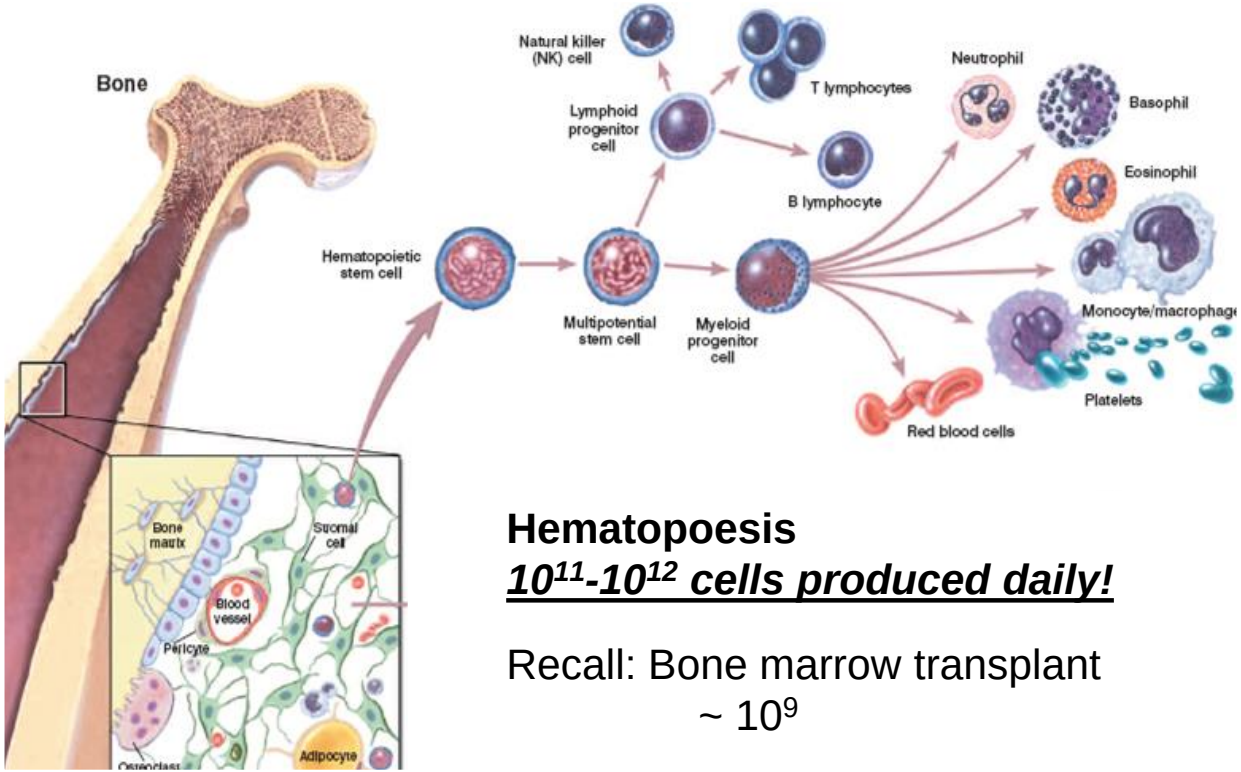


Cell and Tissue Engineering

Cell Numbers, Growth and Kinetics

Part 2 Cell Culture Expansion

Cell proliferation



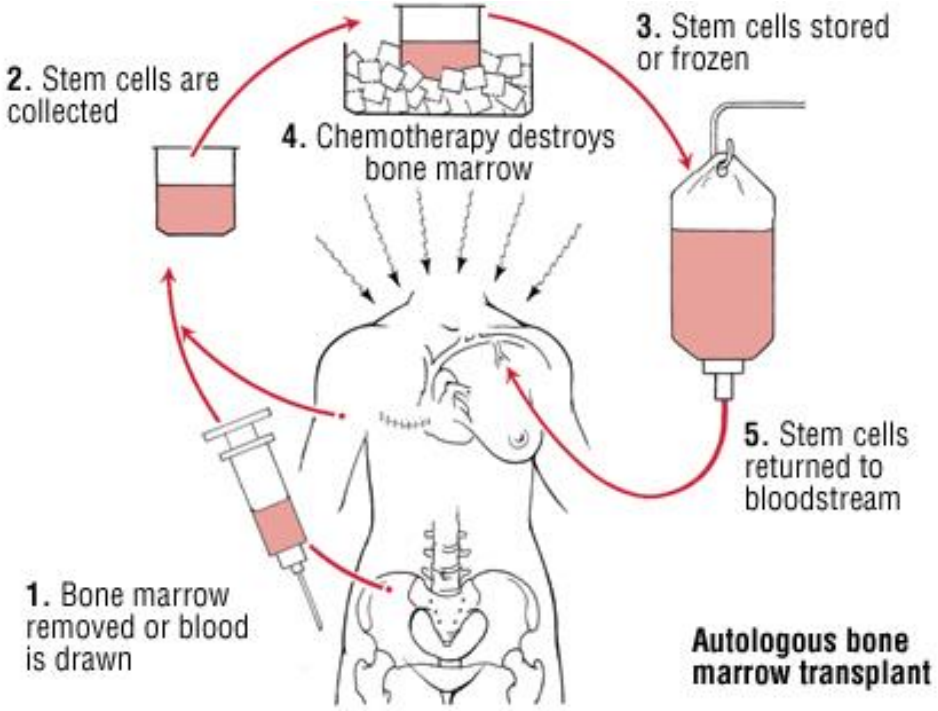
Hematopoesis

<u>10^{11}-10^{12} cells produced daily!</u>

Recall: Bone marrow transplant

~ 10^9

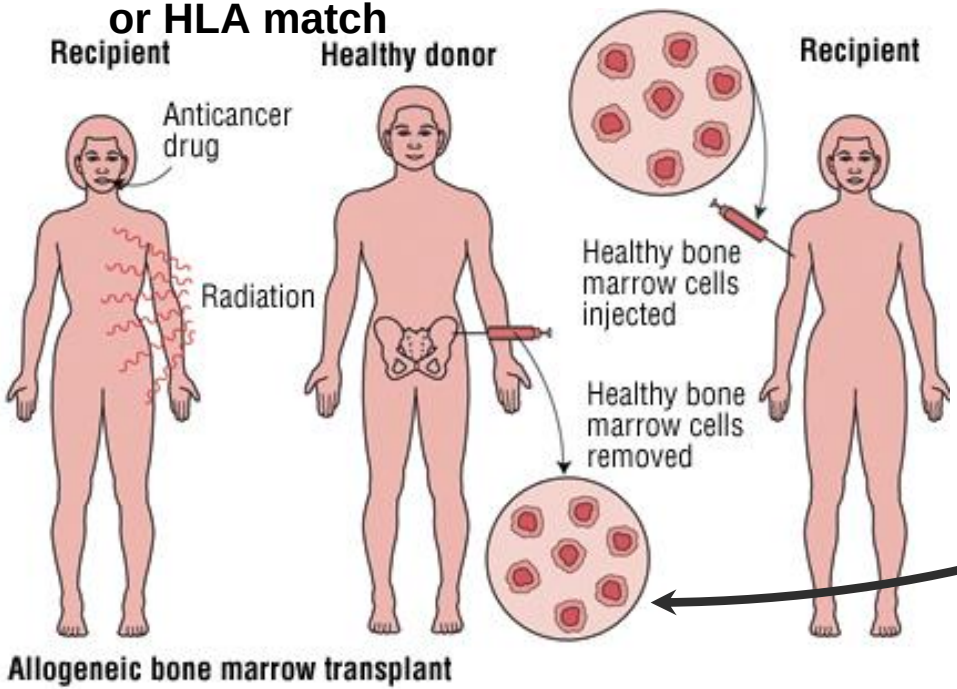
Autologous bone marrow transplants



www.tulane.edu
Wikipedia.org

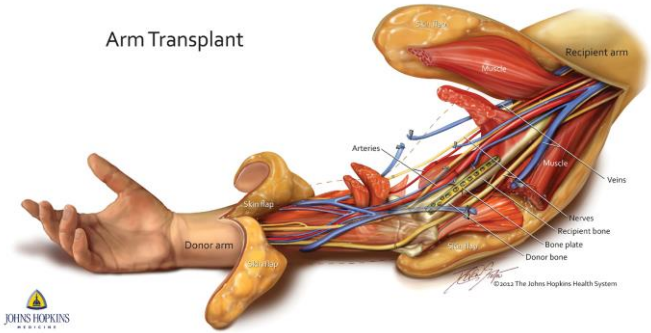
Allogenic bone marrow transplants

Human Leukocyte Antigen or HLA match



Speaking of bone marrow matches…

Quickly recall the double arm transplant we saw in Module 1…

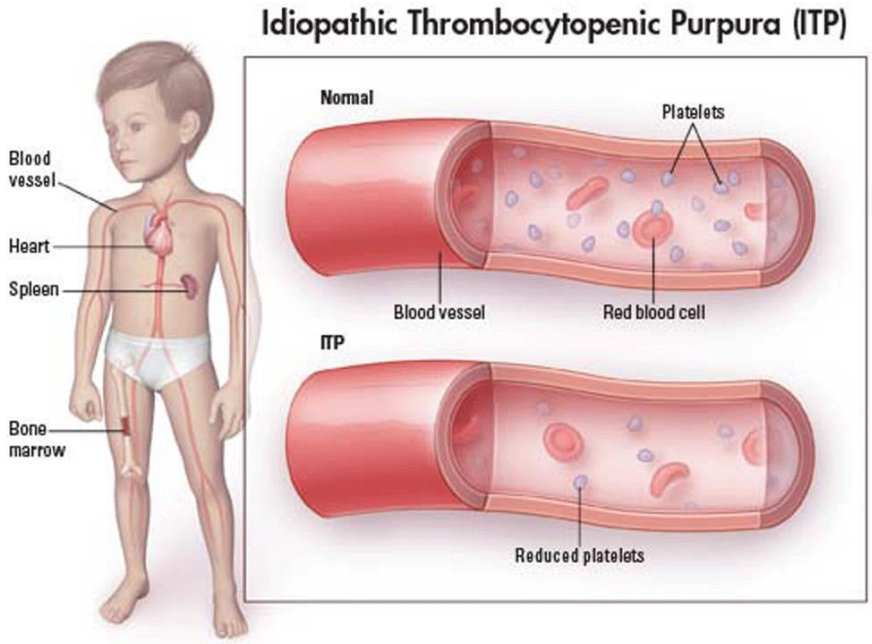


Bone marrow infusion from donor used to reduce rejection risk

Take the case of thrombocytopenia

Back to more traditional bone marrow transplants now…

- Umbilical cord blood (UBC) is one source of replacement cells are an option when bone marrow matches are unavailable

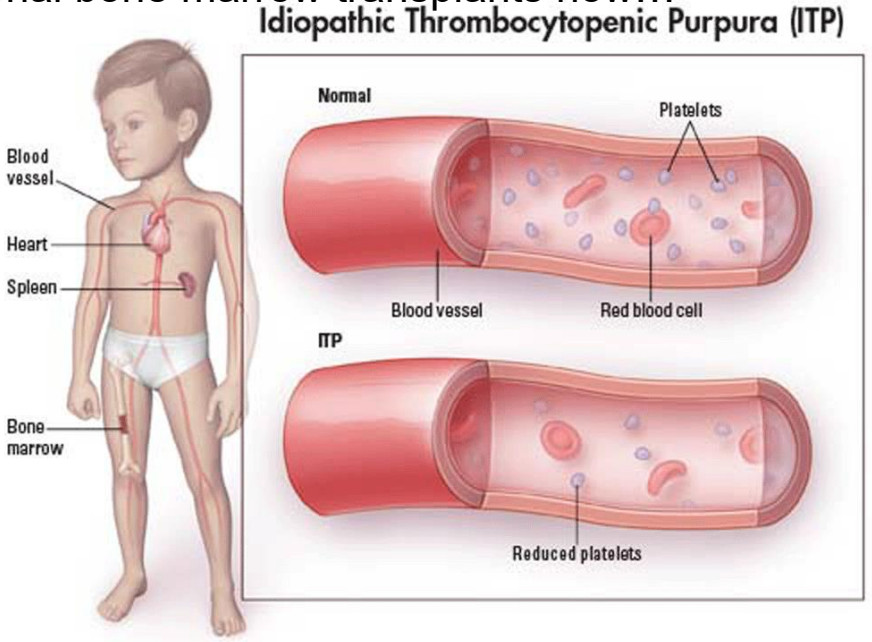


Take the case of thrombocytopenia

Back to more traditional bone marrow transplants now…

- Umbilical cord blood (UBC) is one source of replacement cells are an option when bone marrow matches are unavailable

- Long-term success of this treatment hinges on high numbers of cell
  - Requires *ex vivo* expansion of cells



What can we do ex vivo?

Expand cell in culture!

But this has limits!

Telomeres limit cell divisions

The number of divisions that a primary cell can undergo is subject to the *Hayflick limit:* 30-50 divisions in culture

“Mitotic Clock”

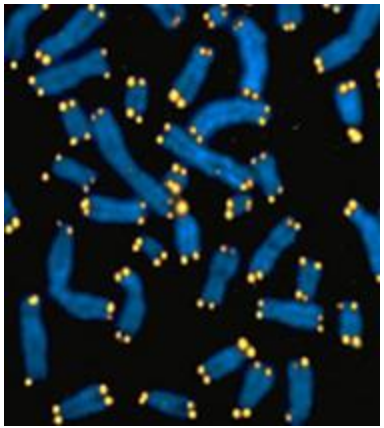
Prweb.com
Ucsf.edu

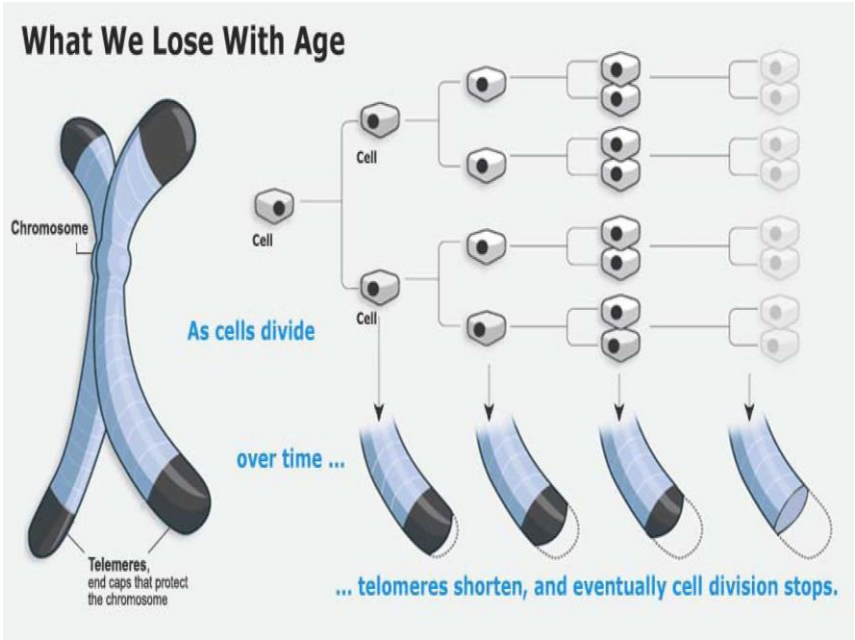
Telomeres limit cell divisions

“Mitotic Clock”

The number of divisions that a primary cell can undergo is subject to the *Hayflick limit:* 30-50 divisions in culture

Telomeres





Prweb.com
Ucsf.edu

Telomeres limit cell divisions

“Mitotic Clock”

Telomerase rebuilds the telomeres

Low activity – insufficient bone marrow replication, anemia

High activity – in 80-90% of cancers allow cancer cells to escape cell cycle arrest

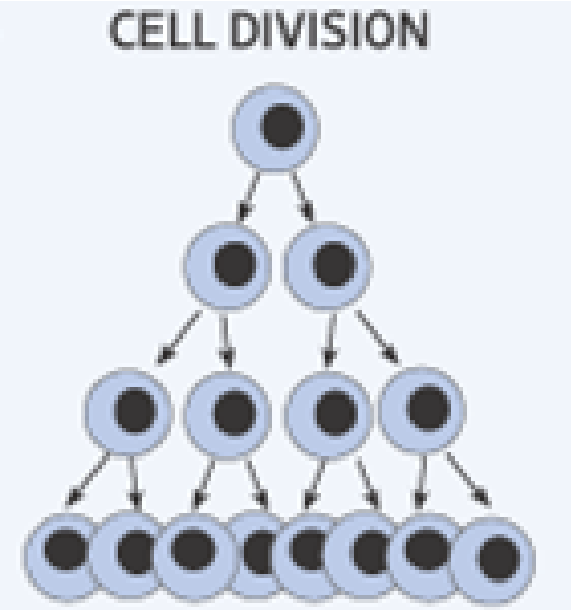
Anti-aging???

Commons.Wikimedia.org
Nobelprize.org

What does the Hayflick limit do to our meaningful cell numbers?

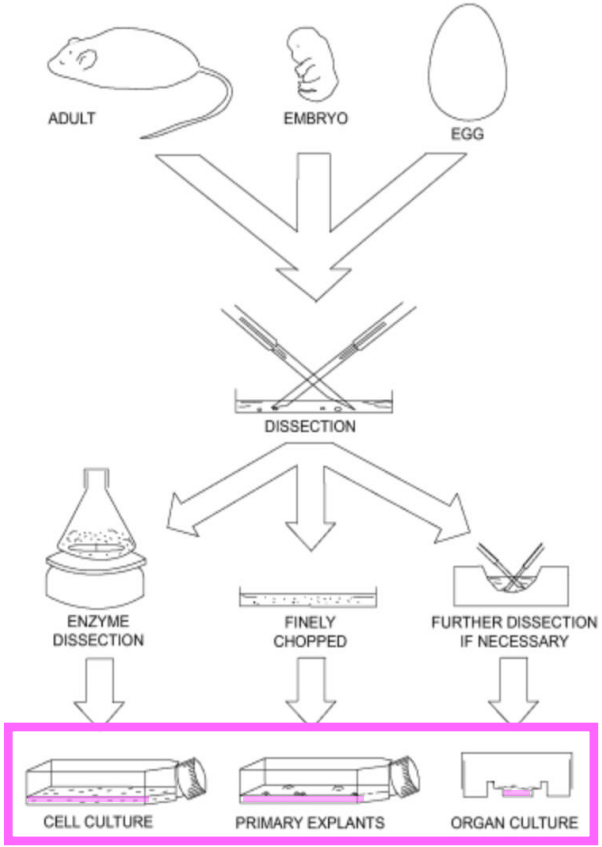
1 cell in culture can give rise to 10^{10} -10^{15} cells

Typical cellular therapies require 10^{7}-10^{9} cells



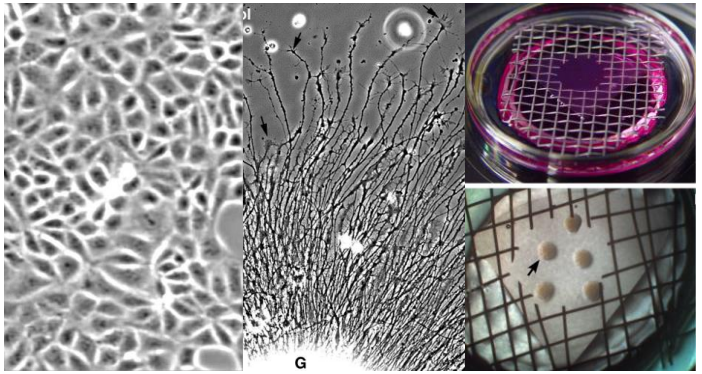
Aging Ills Reversed in Mice, Naik, WSJ

Major methods for obtaining cells from tissues



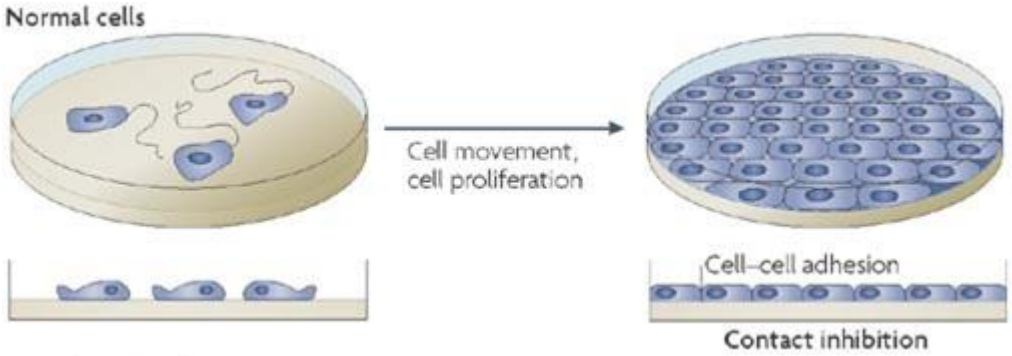
Cell/Tissue Culture – growth of cells/tissue separate from the organism

Cell Culture *Explant Culture* *Organ Culture*



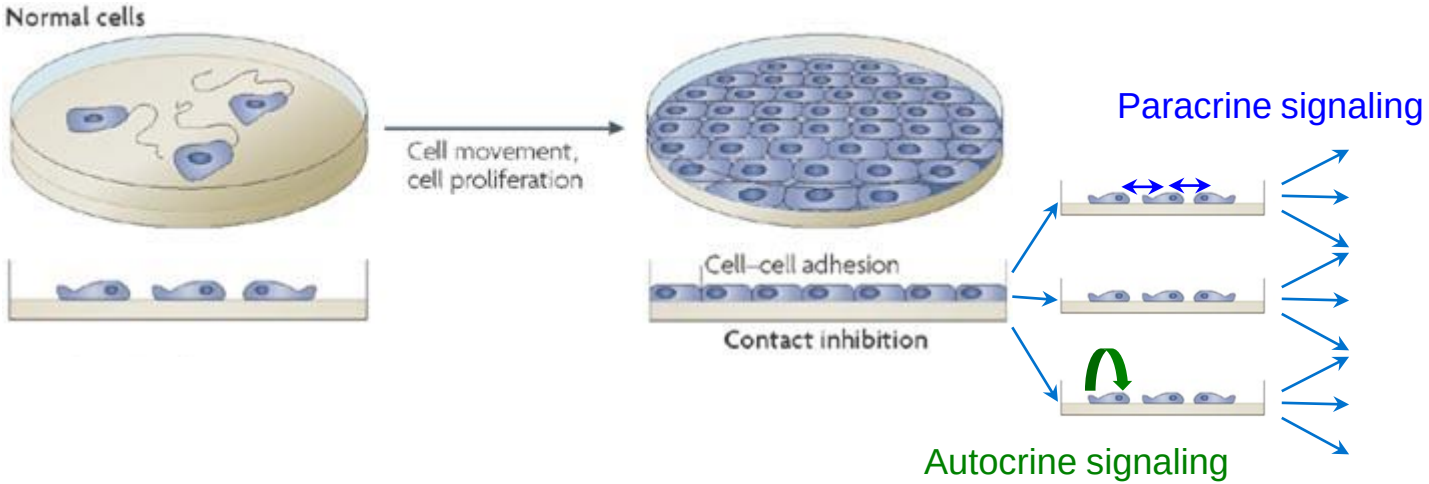
Tissue Engineering, Saltzman
Owen and Weeks, 2003
Ibidi.com ,Wikipedia.org
Cshprotocols.cshlp.org

Contact and density-dependent inhibition of cell growth

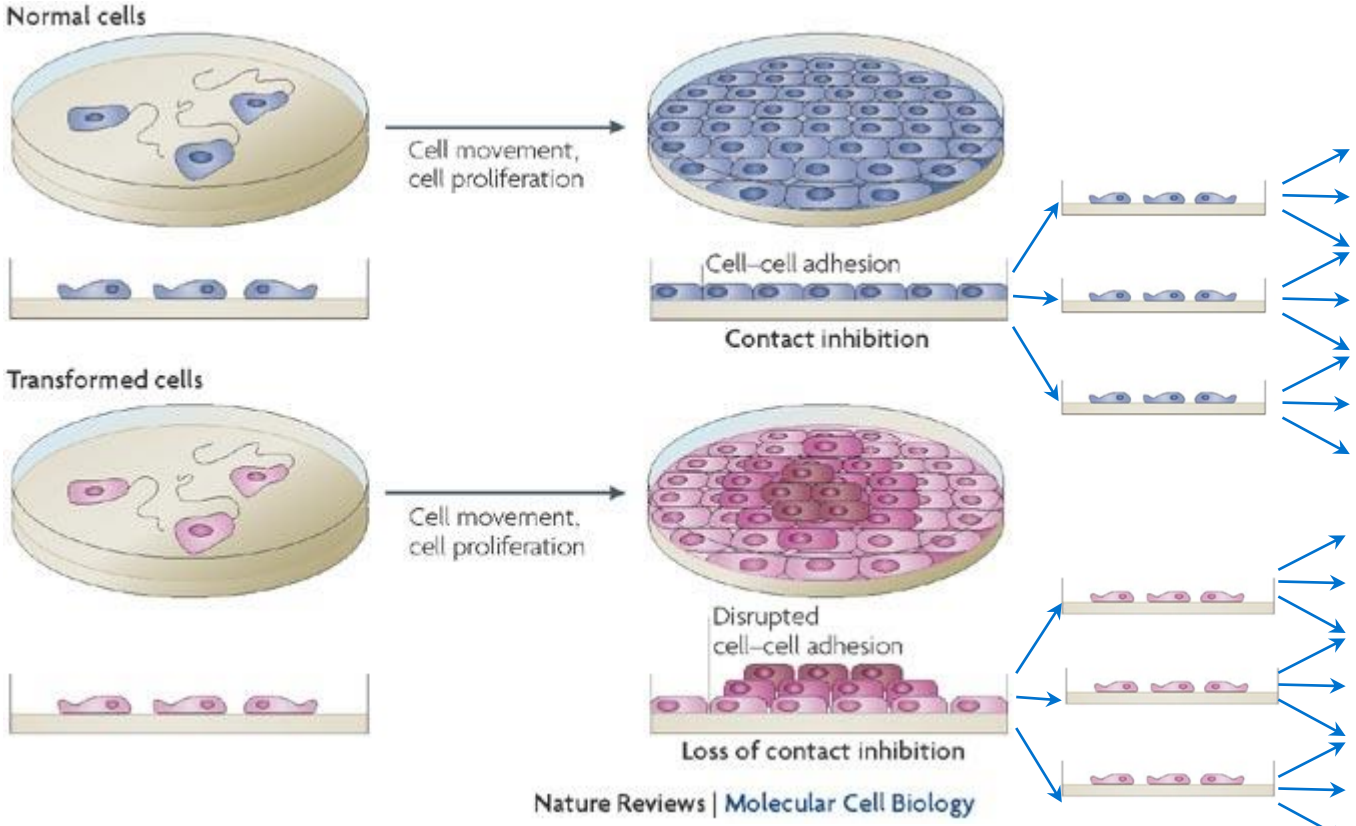


Takai et al, Nature Reviews, 2008

Contact and density-dependent inhibition of cell growth



Contact and density-dependent inhibition of cell growth



Splitting or passaging cell allows for expansion

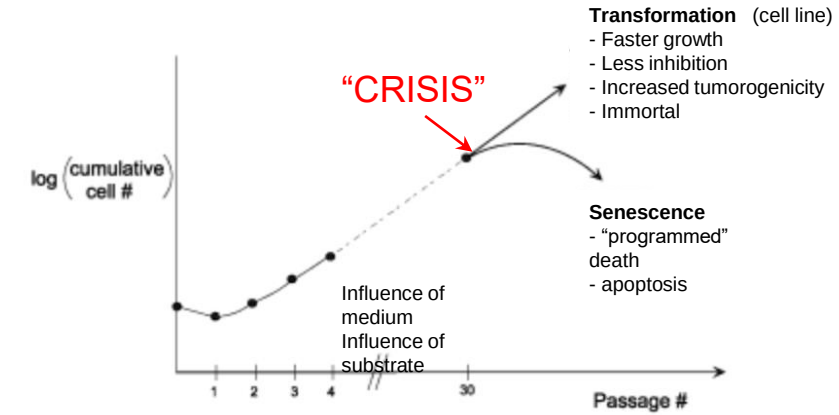
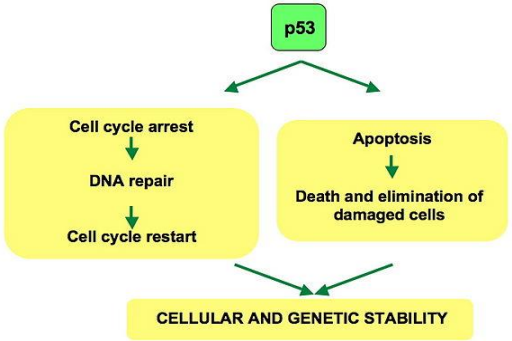
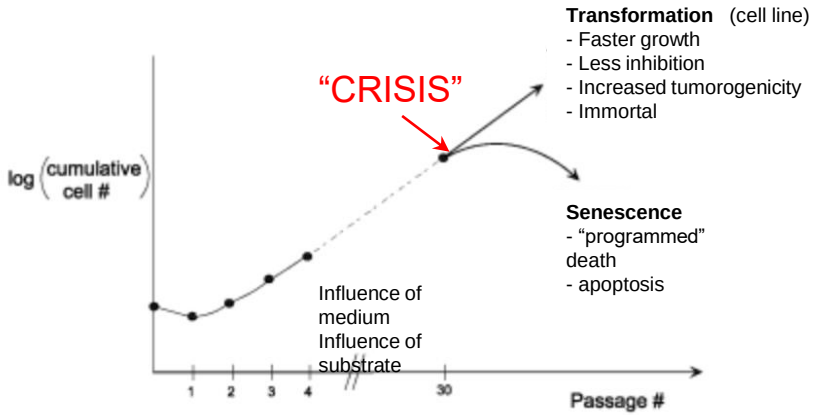


Figure 4.21. Increase of cell number with sequential culture (redrawn from Freshney and Liss [22]). Sequential passaging of cells leads to an increase in the cumulative cell number (i.e., the total number of cell progeny produced from the initial starting material). As the length of time in culture increases, the properties of the cells change.



Splitting or passaging cell allows for expansion



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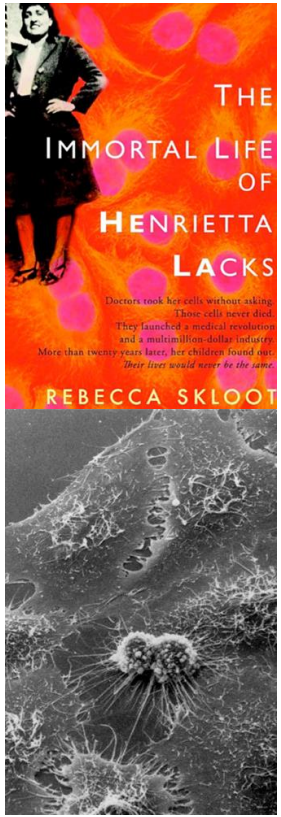


HeLa cells are immortal

Cell culture requires complex medium

Vitamins
Amino acids
Glucose
Salts
…

When minimum essential nutrients are unknown ***serum*** from other animals is used

Serum – non-cellular, non-clotting fraction of blood. Contains hormones and growth factors

Antibiotic are used to keep the cultures free of bacterial contamination

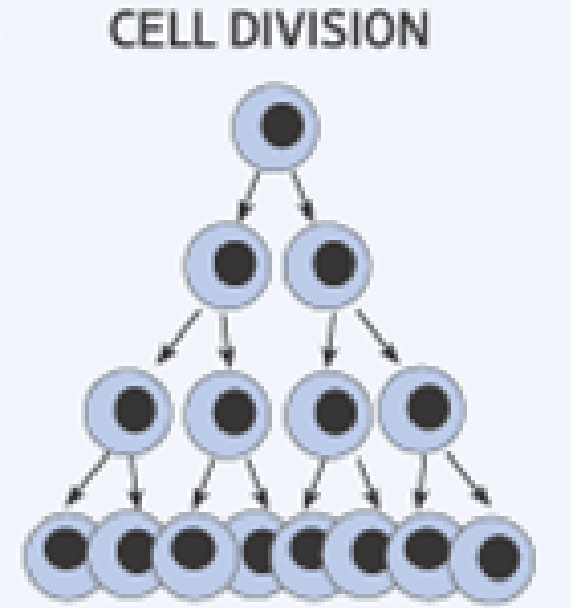
Cell Growth Kinetics

Back to math!

$$\frac{dN}{dt} = k_p \cdot N$$

N is the total number of cells
K_p is the rate constant for cell proliferation
t is time

Cell Growth Kinetics

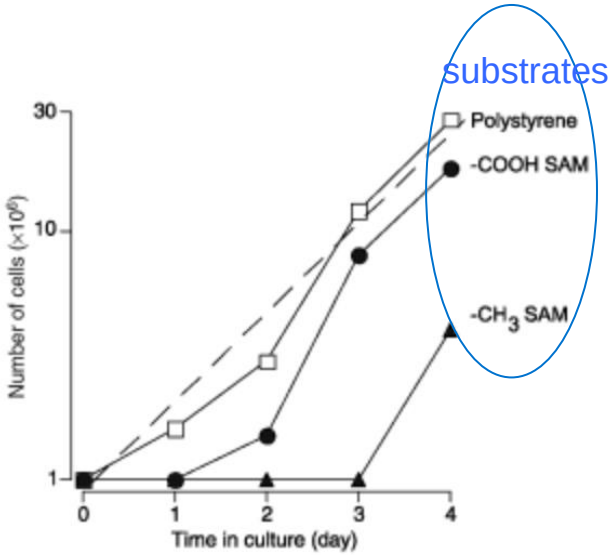


$$\frac{dN}{dt} = k_p \cdot N$$

N is the total number of cells
K_p is the rate constant for cell proliferation
t is time
N_0 is the number of cells at time = 0

$$N = N_0 \cdot e^{k_p \cdot t}$$

Cell Growth Kinetics



Substrates influence growth rates and require more complex mathematical models

$$\frac{dN}{dt} = k_p \cdot N$$

N is the total number of cells
K_p is the rate constant for cell proliferation
t is time
N_0 is the number of cells at time = 0

$$N = N_0 \cdot e^{k_p \cdot t}$$

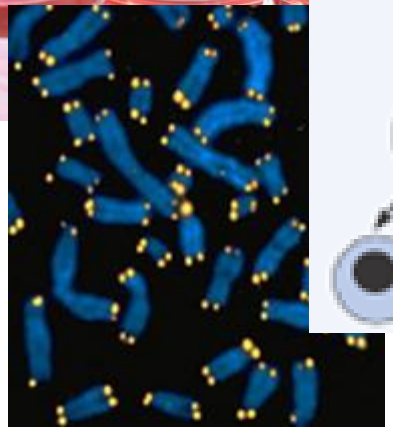
t_D is the doubling time

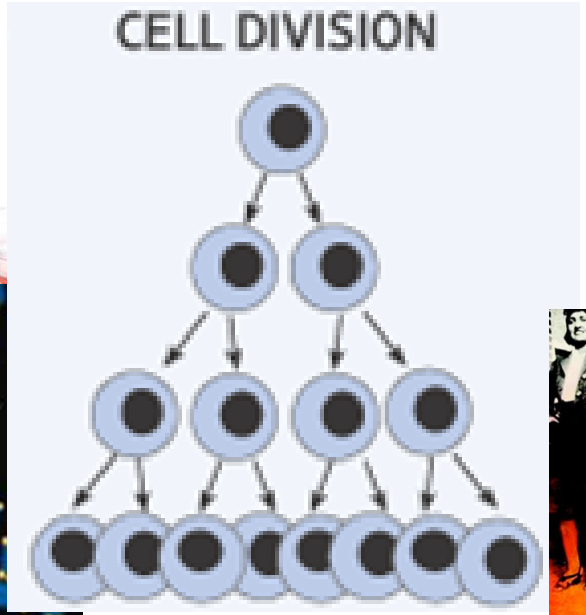
$$N = 2N_0$$

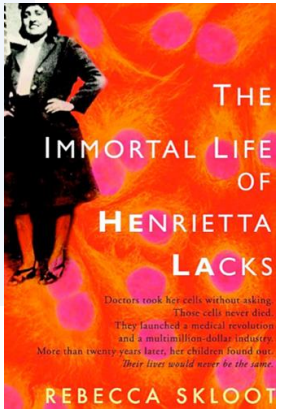
$$t_D = \frac{\ln 2}{k_p}$$

Do the math yourself!

Review and Rewind

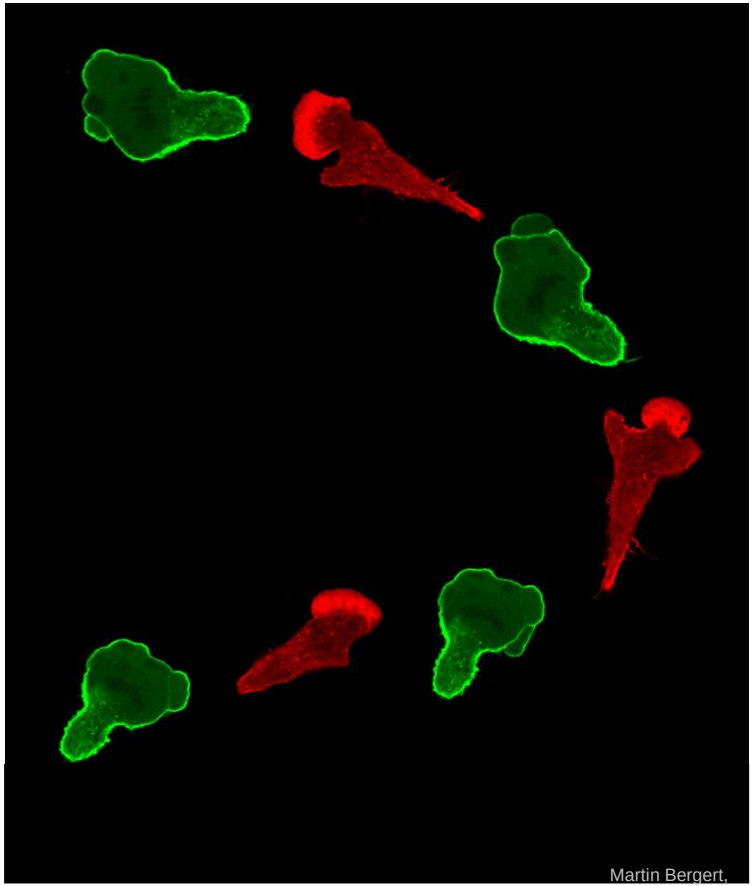






Next Module

- Cell Adhesion and Migration



Martin Bergert,
Max Planck Institute for Molecular Cell Biology and Genetics