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Centro E. Piaggio
bioengineering and robotics research center

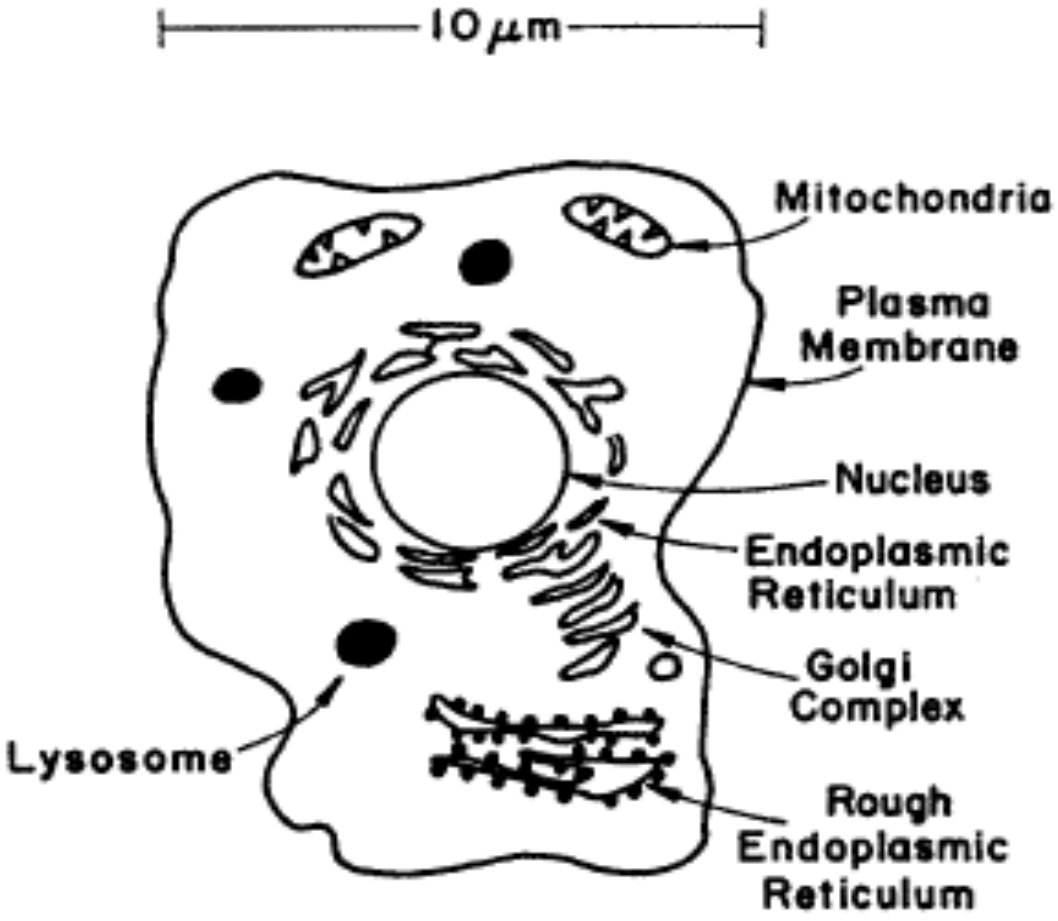

UNIVERSITAS STUDII PADUANI
MCCXXII

Cells

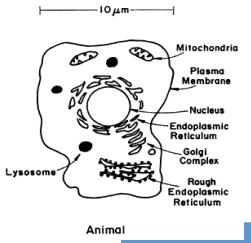
Eukaryotes: Animal cell

- Animal cells are eukaryotic cells, or cells with a membrane-bound nucleus.
- contain other membrane-bound organelles, or tiny cellular structures, that carry out specific functions necessary for normal cellular operation.

Figure 1: structure of animal cell

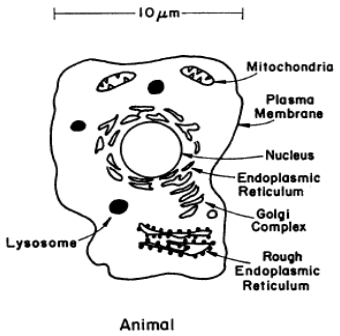


Animal



Structure and function of organelles

Organelles	Function
Nucleus	• Regulate synthesis of proteins in cytoplasm through mRNA • Nucleolus- site of ribosome synthesis • Chromosome: nuclear material (DNA)
Plasma membrane	• selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells
Smooth Endoplasmic reticulum	• Lipid synthesis
Rough endoplasmic reticulum (with ribosome)	• Critical in protein synthesis and posttranslational processing
Mitochondria	• Site of respiration and production of ATP.
Golgi complex	• Completion of complex glycosylation • Collecting and secreting extracellular proteins • Directing intracellular protein traffic to other organelles.



Animal

Lysosomes	• **Contain hydrolytic enzymes (proteases, nucleases and esterases)** • **Digestion of certain food particles ingested by the cell**
Peroxisomes and glyoxysomes	• Perixidases (hydrolysis of H_2O_2) • Glyoxalases (glyoxylic acid metabolism
Cytoskeleton	• Provide cell mechanical strength and control its shape • critical in cell movement • Transduction of mechanical forces into biological responses • Separation of chromosomes into two daughter cells during cell division

Characteristic of animal cell

- Size: between 10-30 µm.
- Shape: (spherical or ellipsoidal).
- Cell structure:

a) Surrounded by thin and fragile plasma membrane.
b) Has microvilli- to increase surface area.
c) Surface of the cell is negatively charged and cells tend to grow on positively charge surface (for anchorage-dependent cells).
d) Posses specific cell surface receptors that adhere to ligand on the surface.

Characteristic of animal cell

- Some animal cells are non-anchorage dependent and grow in suspension culture.
- Has cytoskeleton or system of protein filaments (actin filaments, intermediate filaments and microtubules)-provide cell mechanical strength, control shape and guide cell movement.
- Some animal cells contain cilia- used to transport substrate across the cell surface.

Classes of Culture Cells

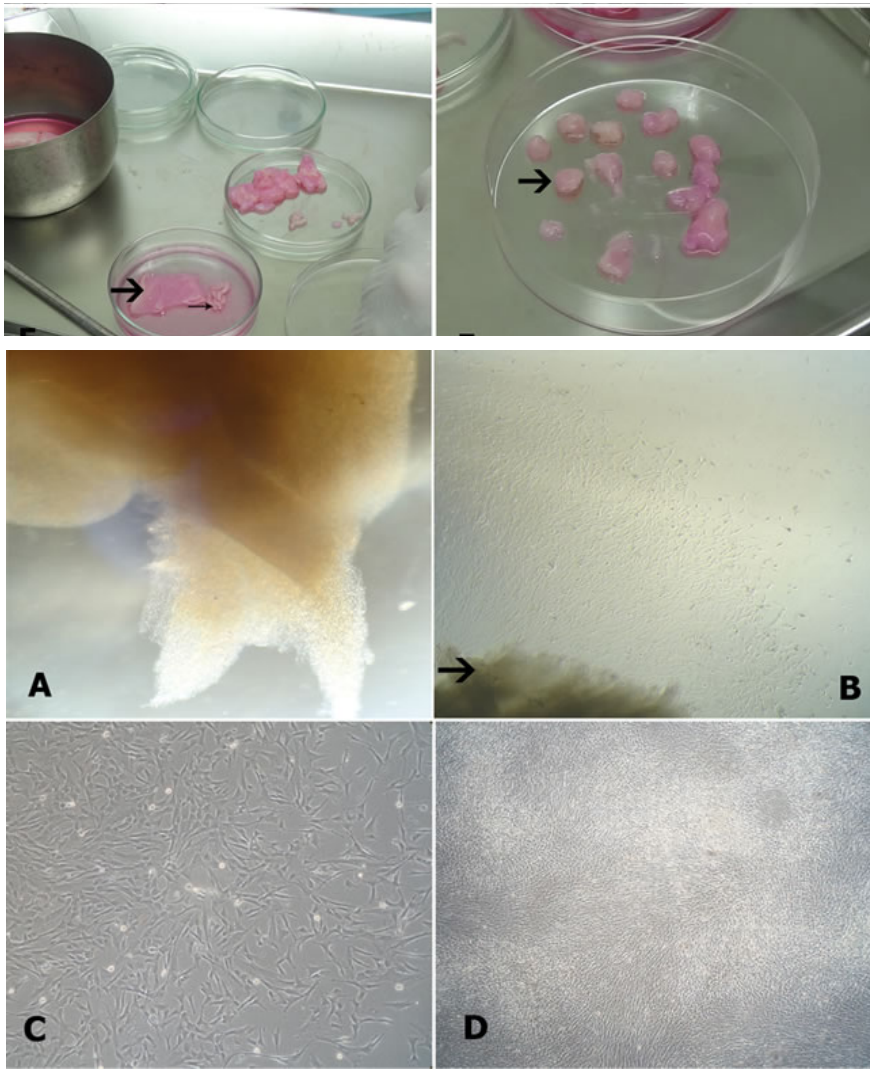
- Cultures of animal cells are usually divided into 3 classes:
  1. Primary cells
  2. Cell strains
  3. and cell lines

1- Primary Culture

- When cells are surgically removed from an organism and placed into a suitable culture environment they will attach, divide and grow
- Most of the primary culture cells have a finite lifespan of 5-10 divisions in vitro
- Due to their limited lifespan, one cannot do long-term experiments with these cells
- Primary cells are considered by many researchers to be more physiologically similar to in vivo cells

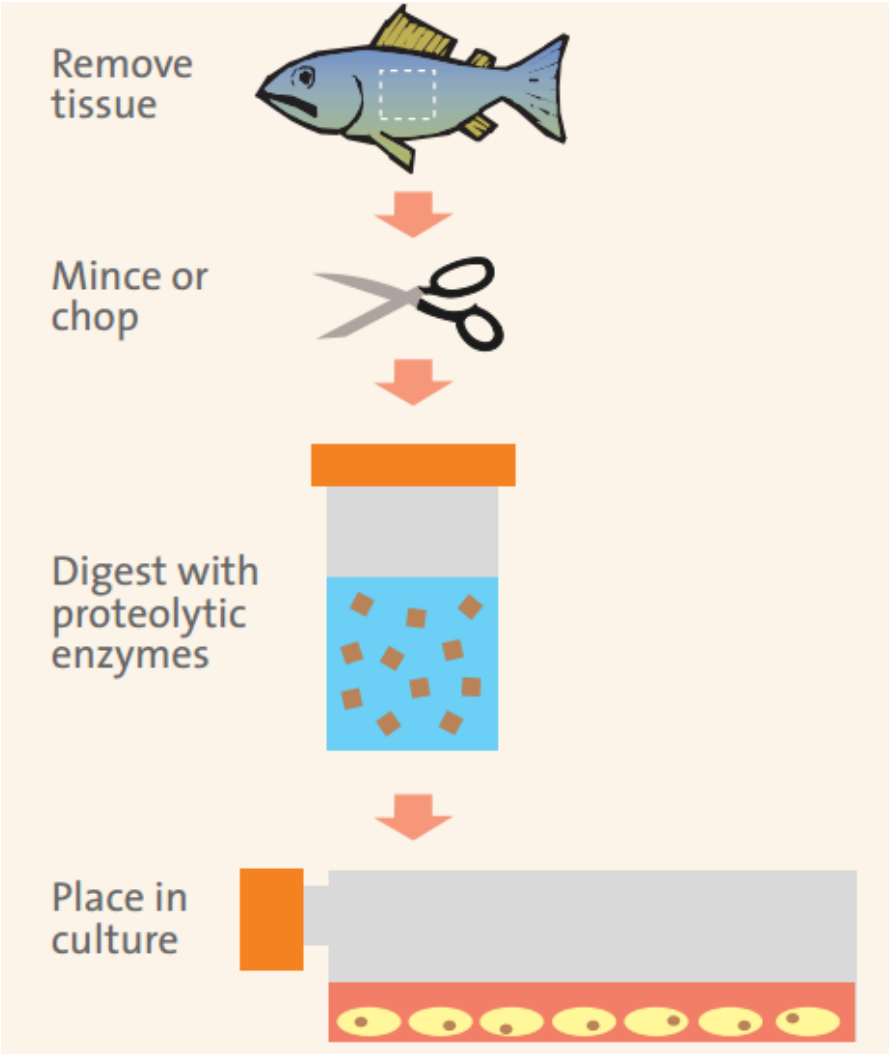
1- Primary Culture

- There are two basic methods for obtaining primary culture:
  1. **Explant cultures:**
     - Small pieces of tissue are attached (using plasma clots or fibrinogen) to a glass or treated plastic culture vessel and immersed in culture medium
     - After a few days individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow



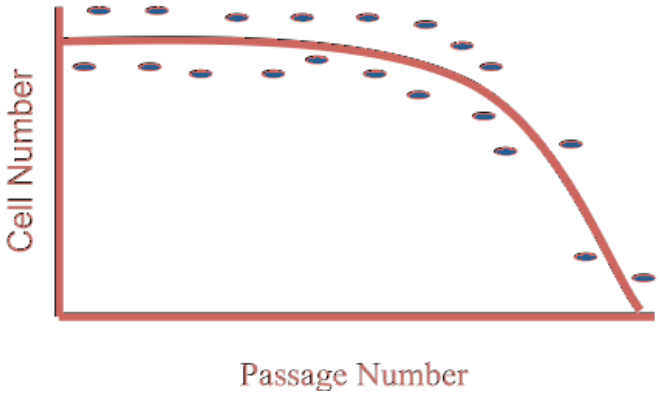
1- Primary Culture

2. Enzymatic dissociation:
   - More widely used
   - speeds up the process by adding digesting (proteolytic) enzymes such as trypsin or collagenase to the tissue fragments to dissolve the cement holding the cells together
   - This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide



Hayflick's Phenomenon

- Cells will continue to grow and divide normally for a limited number of passages
- When they get to a certain point even if they are given the appropriate nutrients, they simply stop dividing and will eventually die
- There appears to be a correlation between the maximal number of passages and aging
- The number of passages decreases when cells are harvested from older individuals



2- Cell Strains

- Cell strains are cells that have been adapted to culture but, unlike cell lines, have a finite division potential
- Upon serial transfers of primary cells, a gradual selection may occur until a particular cell type becomes predominant
- If these cells continue to grow at a constant rate over successive passages, these primary cells are referred to as a cell strain
- These cells have a finite lifespan of 40-60 divisions in vitro
- They are useful in vaccine production

3- Cell Lines

- If the cells in a cell strain undergo a transformation process (spontaneous or induced changes in karyotype, morphology or growth properties) that makes them "immortal“ (able to divide indefinitely) they are called a cell line
- It is not known how a diploid cell strain becomes a cell line, although this event may be mimicked by infection with oncogenic viruses or by exposure to chemical carcinogens
- Cell Lines often have abnormal chromosome numbers and maybe tumorigenic when inoculated into susceptible animals
- Cell lines that have been derived from tumors often do not exhibit contact-inhibition (inhibition of growth under crowded conditions), but rather continue to pile-up

Transformation of Cells

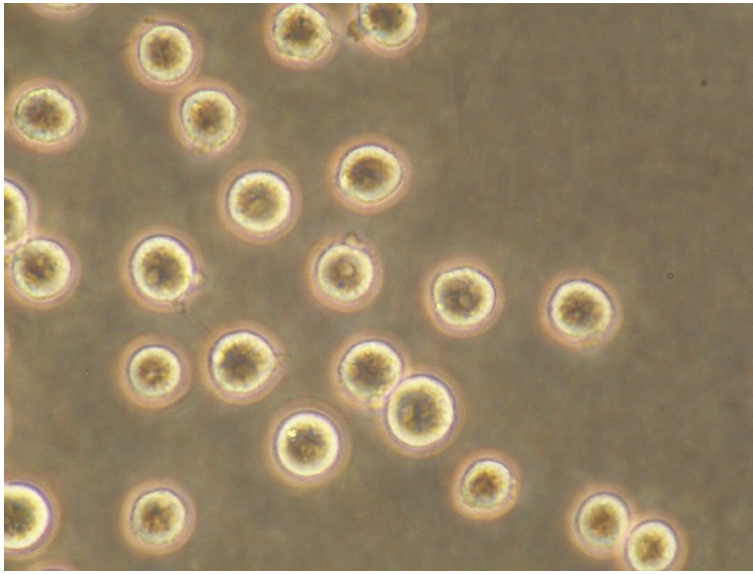
- Transformed, Infinite or Established Cells
- Changed from normal cells to cells with many of the properties of cancer cells
- Some of these cell lines have actually been derived from tumors or are transformed spontaneously in culture by mutations
- Chemical or gamma ray treated cells can become infinite with loss of growth factors
- Viral infection with SV40 T antigen can insert oncogenes and lead to gene alteration
- No matter how transformation occurred, the result is a cell with altered functional, morphological, and growth characteristics

Cell Culture Systems

- Cells may be loosely divided into two main types:

  1- Suspension cell culture (Anchorage-independent)

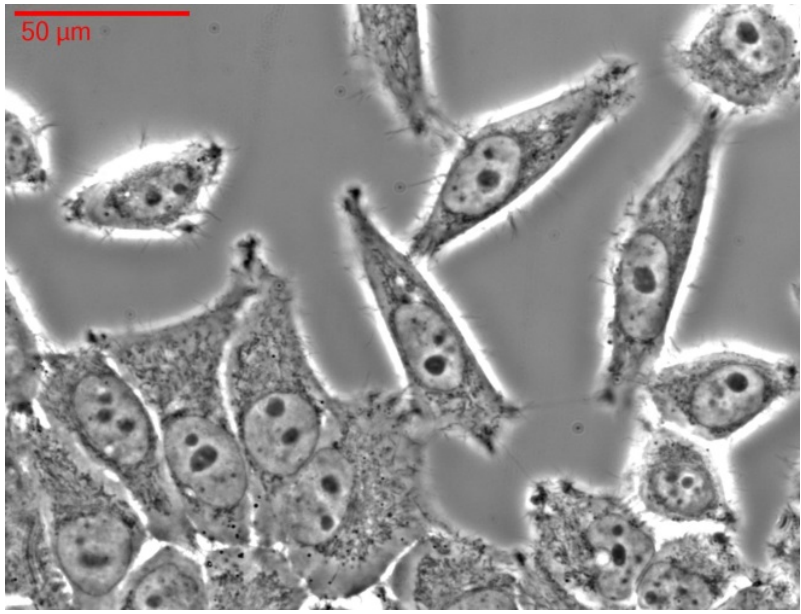
  - derived from cells which can divide and survive without being attached to a substrate,
  - e.g. cells of haemopoietic lineage
  - Can be maintained in culture vessels that are not tissue-culture treated,
  - requires agitation for adequate gas exchange
  - Easier to passage



Cell Culture Systems

2- Adherent cell culture (Anchorage-dependent)

- must adhere to a surface to survive
- Form monolayers
- e.g. cells derived from different tissue (breast, liver)
- Growth is limited by surface area
- Will cease proliferating once they become confluent (completely cover the surface of cell culture vessel)
- Cells are dissociated enzymatically or mechanically from surface



Growth Cycle in Attachment Culture

- Eukaryotic cells in attachment culture have a characteristic growth cycle similar to bacteria
- The growth cycle is typically divided into three phases:

1- Lag Phase

  - This is the time following subculture and reseeding during which there is little evidence of an increase in cell number
  - It is a period of adaptation during which the cell replaces elements lost during trypsinization, attaches to the surface, and spreads out

Growth Cycle in Attachment Culture

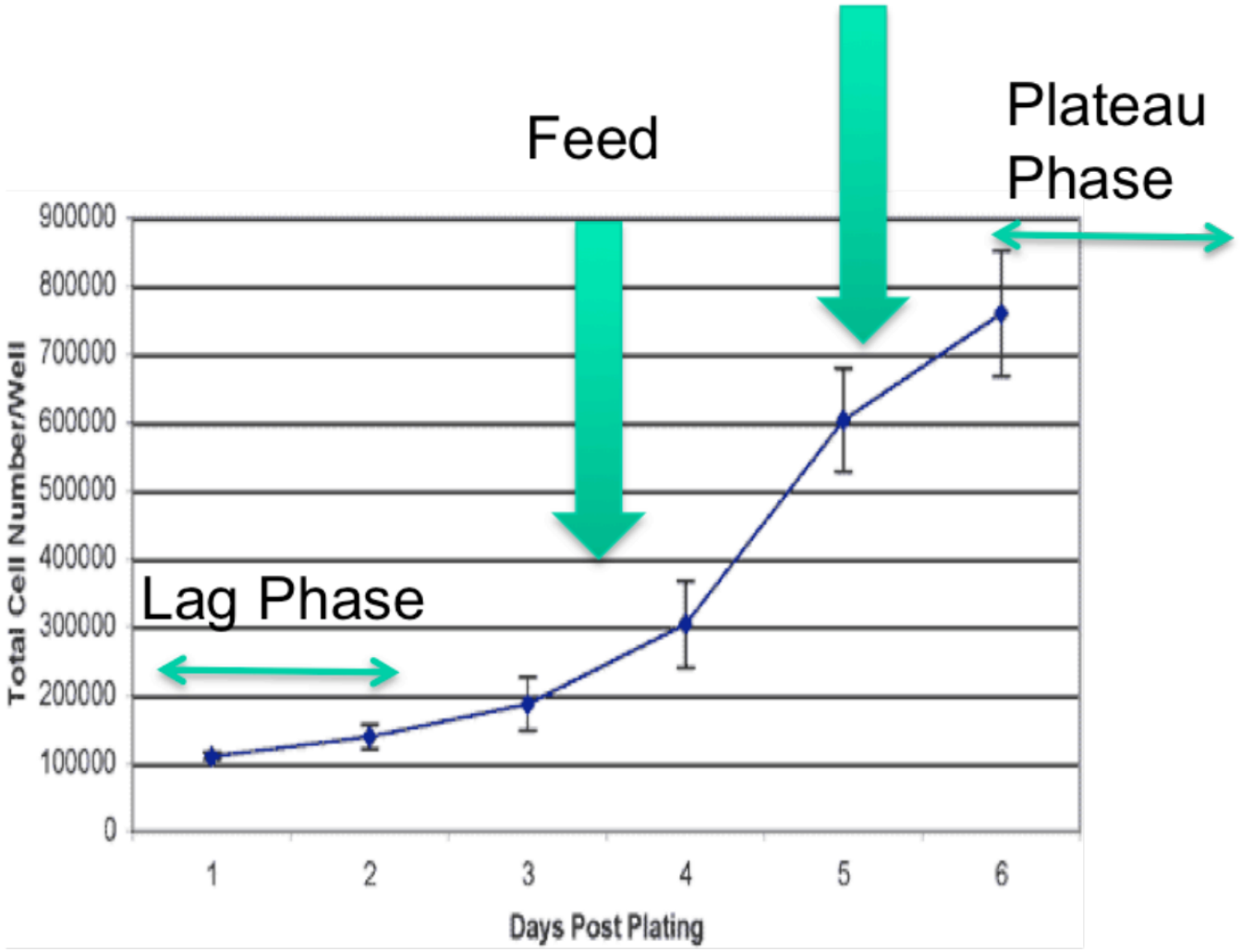
2- Log Phase

- This is the period of exponential increase in cell number
- The length of the log phase depends on the seeding density, the growth rate of the cells
- It is the optimal time for sampling since the population is at its most uniform and viability is high

3- Plateau Phase

- Toward the end of the log phase, the culture becomes confluent
  - All the available growth surface is occupied and all the cells are in contact with surrounding cells
- Following confluence the growth rate of the culture is reduced, and in some cases, cell proliferation ceases almost completely
- At this stage, the culture enters the plateau (or stationary) phase, and the growth fraction falls

Growth Cycle in Attachment Culture

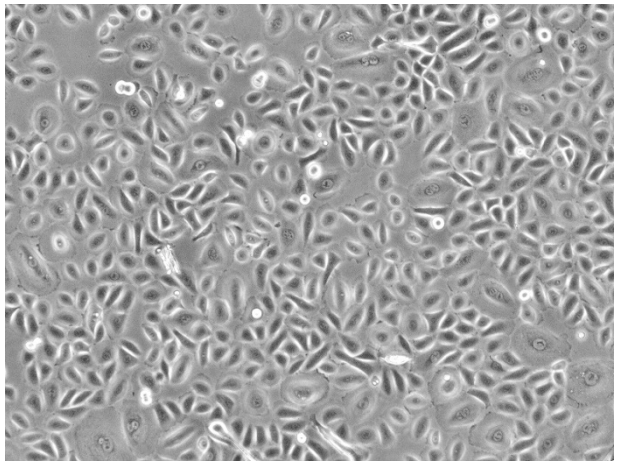


Morphology of Cells

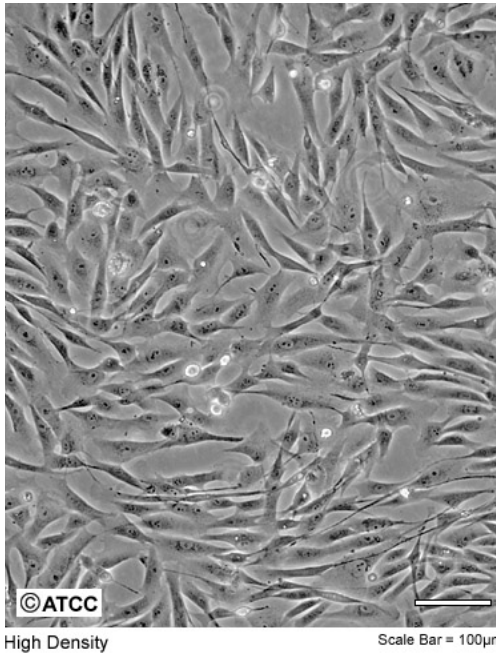
- Cultured cells are usually described based on their morphology (shape & appearance), there are two basic morphologies:

1. Epithelial-like:
   - cells that appear flattened and polygonal in shape
2. Fibroblast-like:
   - cells that appear thin and elongated

- Culture conditions paly an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies



Human Conjunctival Epithelial Cells (HConEpiC) - Phase contrast, 100x.



***Homo sapiens*, human, Foreskin**

Basic Requirements For Successful Cell Culture

1. The first necessity is a well-established and properly equipped cell culture facility. All facilities should be equipped with the following:
   - A certified biological safety cabinet
     - protects both the cells in culture and the worker from biological contaminants
   - A centrifuge, preferably capable of refrigeration
   - A microscope for examination of cell cultures and for counting cells
   - And a humidified incubator set at 37°C with 5% CO_2 in air
   - A 37°C water bath filled with water containing inhibitors of bacterial and fungal growth can also be useful if warming of media prior to use is desired

Basic Requirements For Successful Cell Culture

2. The second requirement for successful cell culture is the practice of sterile technique
   - Prior to beginning any work, the biological safety cabinet should be turned on and allowed to run for at least 15 min to purge the contaminated air
   - All work surfaces within the cabinet should be decontaminated with an appropriate solution;
     - 70% ethanol or isopropanol are routinely used for this purpose
   - Any materials required for the procedure should be similarly decontaminated and placed in or near the cabinet
   - This is especially important if solutions have been warmed in a water bath prior to use
   - The worker should put on appropriate personnel protective equipment for the cell type in question

Basic Requirements For Successful Cell Culture

- Gloved hands should be sprayed with decontaminant prior to putting them into the cabinet and gloves should be changed regularly if something outside the cabinet is touched
- Care should be taken to ensure that anything coming in contact with the cells of interest, or the reagents needed to culture and passage them, is sterile (either autoclaved or filter-sterilized)

Basic Requirements For Successful Cell Culture

3. A third necessity for successful cell culture is appropriate, quality controlled reagents and supplies
   - There are numerous suppliers of tissue culture media and supplements
   - Examples include:
     - Invitrogen (www.invitrogen.com),
     - Sigma–Aldrich (www.sigmaaldrich.com),
     - BioWhittaker (www.cambrex.com),
     - and StemCell Technologies Inc. (www.stemcell.com).
   - Similarly, there are numerous suppliers of the plasticware needed for most cell culture applications (i.e., culture dishes and/or flasks, tubes, disposable pipets)

Basic Requirements For Successful Cell Culture

4. The final necessity for successful cell culture is the knowledge and practice of the fundamental techniques involved in the growth of the cell type of interest
   - The majority of cell culture carried out by investigators involves the use of various non-adherent or adherent continuously growing cell lines
   - These cell lines can be obtained from reputable suppliers such as:
     - the American Tissue Type Collection (ATCC; www.atcc.org)
     - or DSMZ (the German Collection of Microorganisms and Cell Cultures) (www.dsmz.de/mutz/mutzhome.html)
     - Alternatively, they can be obtained from collaborators
   - Regardless of the source of the cells, it is advisable to verify the identity of the cell line and to ensure that it is free of mycoplasma contamination

Cell Culture Medium

- Cells have complex nutritional requirements that must be met to permit their propagation in vitro
- Different types of cells have different growth requirements and a number of chemically-defined formulations have been developed that support the growth of a variety of established cell lines
- Although some serum-free media are available and some cell lines have been adapted to growing in such a medium, most cell lines require the addition of 5-10% serum as a supplement to promote cellular multiplication
- Fetal Bovine Serum (FBS) is often the best to use

Cell Culture Medium

1- The various nutrients required are:
- glucose,
- fats and fatty acids,
- lipids, phospholipids and sulpholipids,
- ATP and amino acids
- Vitamins
- Minerals

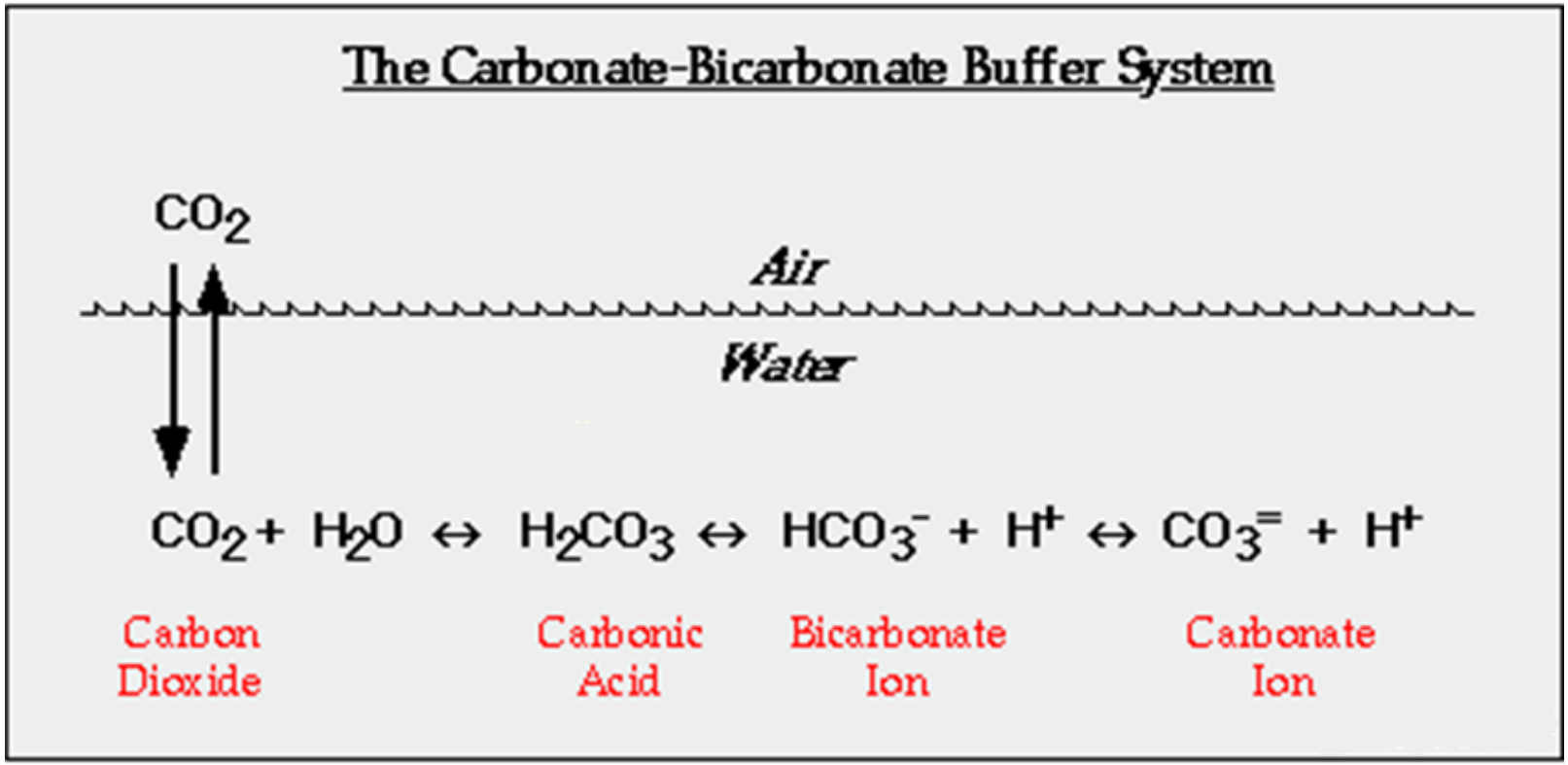
2- Serum:
- Serum can provide various growth factors, hormones and other factors needed by the most mammalian cells for their long term growth and metabolism

Cell Culture Medium

- L-Glutamine
  - L-Glutamine is an essential amino acid required by virtually all mammalian cells grown in culture
  - It is used for protein production, as an energy source, and in nucleic acid metabolism
  - It is also more labile in liquid cell culture media than other amino acids
  - The rate and extent of L-glutamine degradation are related to storage temperatures, age of the product, and pH

Buffering in Cell Culture

- A pH indicator may be Included in the original formulation to permit direct observation of the pH of the medium
- Optimum pH between 7.2 to 7.4 is generally needed for mammalian cells



Buffering in Cell Culture

- Generally in the cell culture medium pH indicator, commonly phenol red is used to analyze the pH of environment in which cells are growing
- Phenol red is:
  - yellow in acidic medium (pH 6.8
  - tomato red at neutral pH (7.0),
  - red at an alkaline pH (7.4)
  - and blue at increased basicity (pH 7.6)
  - and finally purple at high pH

Supplements to Medium: Antibiotics

- Prevention of contamination by the different microorganisms (bacteria, mycoplasma and fungi) is the most important part of all animal cell culture
- The risk of contamination during culture can be avoided by adding different antibiotics, such as:
  - penicillin (100 U/ml) for bacteria,
  - streptomycin (100 mg/ml) for bacteria,
  - or gentamycin (50mg/ ml) for bacteria,
  - and nystatin (50mg/ml) for fungi and yeast
- The routine use of antibiotics is generally not recommended because:
  - it may lead to a relaxation of aseptic technique
  - resistant microorganisms may develop
  - microbial growth may be controlled but biochemical alteration may be produced

Temperature & Humidity

- Temperature
  - Optimum temperature is also required for the proper growth of the cell
  - The optimum temperature of mammal is 37^{o}C
- Humidity
  - Proper humidity is also essential for cell growth as humidity distribution indirectly also has effect on temperature
  - For cell growth 100% humidity is essential to reduce evaporation

Storage of Medium

- Once prepared, the cell culture medium has to be properly stored
- For long-term storage, it should be frozen without $NaHCO_3$
- On a short-term basis the medium should be kept at 4°C and warmed up to 37°C only for the time necessary to perform a given experiment

Culture Vessels

- Culture vessels provide a contamination barrier to protect the cultures from the external environment while maintaining the proper internal environment
- For anchorage-dependent cells, the vessels provide a suitable and consistent surface for cell attachment
- Other characteristics of vessels include easy access to the cultures and optically clear viewing surfaces

Culture Vessels

- Flasks
  - Plastic flasks are available with a range of growing areas, a variety of shapes, with several different neck designs
  - Flasks surfaces are specially treated for growing anchorage-dependent cells

Description	Growth area (cm^2)	Recommended working volume (mL)	Cell yield*
T-25	25	5 – 10	2.5×10^6
T-75	75	15 – 25	7.5×10^6
T-150	150	30 - 50	15×10^6
T-175	175	35 - 60	17.5×10^6
T-225	225	45 - 75	22.5×10^6

***Cell line dependent. Based upon a density of 1×10^5 cells/cm^2.**

Culture Vess

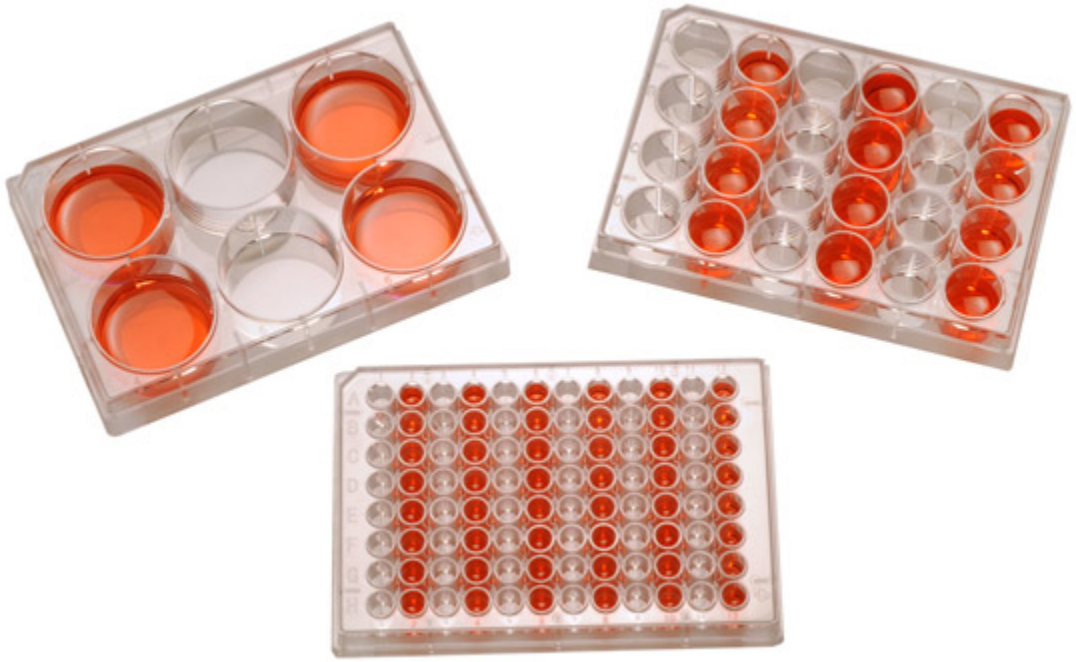
- Cell culture dishes
  - Cell culture dishes offer the best economy and access to the growtl surface
  - Cell culture dishes surfaces are specially treated for growing anchorage-dependent cells

Description	Growth area (cm^2)	Recommended working volume (mL)	Cell yield*
35	8	1 - 2	0.8×10^6
60	21	4 - 5	2.1×10^6
100	55	10 - 12	5.5×10^6
150	148	28 - 32	14.8×10^6

***Cell line dependent. Based upon a density of 1×10^5 cells/cm^2.**

Culture Vessels

- Multiwell plates
  - Multiwell plates offer significant savings in space, media, and reagents when compared to an equal number of dishes



Culture Vessels

- Surface Coatings
- Most tissue culture work uses disposable polystyrene vessels
- The vessel surface is treated to render it hydrophilic
- Most cell lines are cultivated on treated plastic surfaces in dishes or flasks
- Some fastidious cell lines require further treatment of the growth surface before they will attach and proliferate
- The most common techniques include coating the surface with serum, collagen, laminin, gelatin, poly-L-lysine, or fibronectin

Step for removing cell

1. Removal solution for cells : EDTA, TRYPSIN, COLLAGENASE OR PRONASE
2. The exposure time for cell removal : 5-30 min (37°C)
3. After cells are removed from surfaces, serum is added to the culture bottle
4. The serum-containing suspension is centrifuged, washed with buffered isotonic saline solution and used to inoculate secondary culture

Mamalian cells are divided by Normal (mortal) and immortal (continuous/transformed)
Normal: Divide only for limited of generation (30generations)
Transformed: Can be propagated

TABLE 12.1 Comparison of "Normal" and "Transformed" Cells

Normal	Transformed
1. Anchorage-dependent (except blood cells)	1. Nonanchorage-dependent (i.e., suspension culture possible)
2. Mortal; finite number of divisions	2. Immortal or continuous cell lines
3. Contact inhibition; monolayer culture	3. No contact inhibition; multilayer cultures
4. Dependent on external growth factor signals for proliferation	4. May not need an external source of growth factors
5. Greater retention of differentiated cellular function	5. Typically loss of differentiated cellular function
6. Display typical cell surface receptors	6. Cell surface receptor display may be altered

Characteristic:

Contact inhibition: cell division is inhibited when cell's surface is in contact with other cell

No contact inhibition: the cells do not sense the presence of other cells and keep dividing

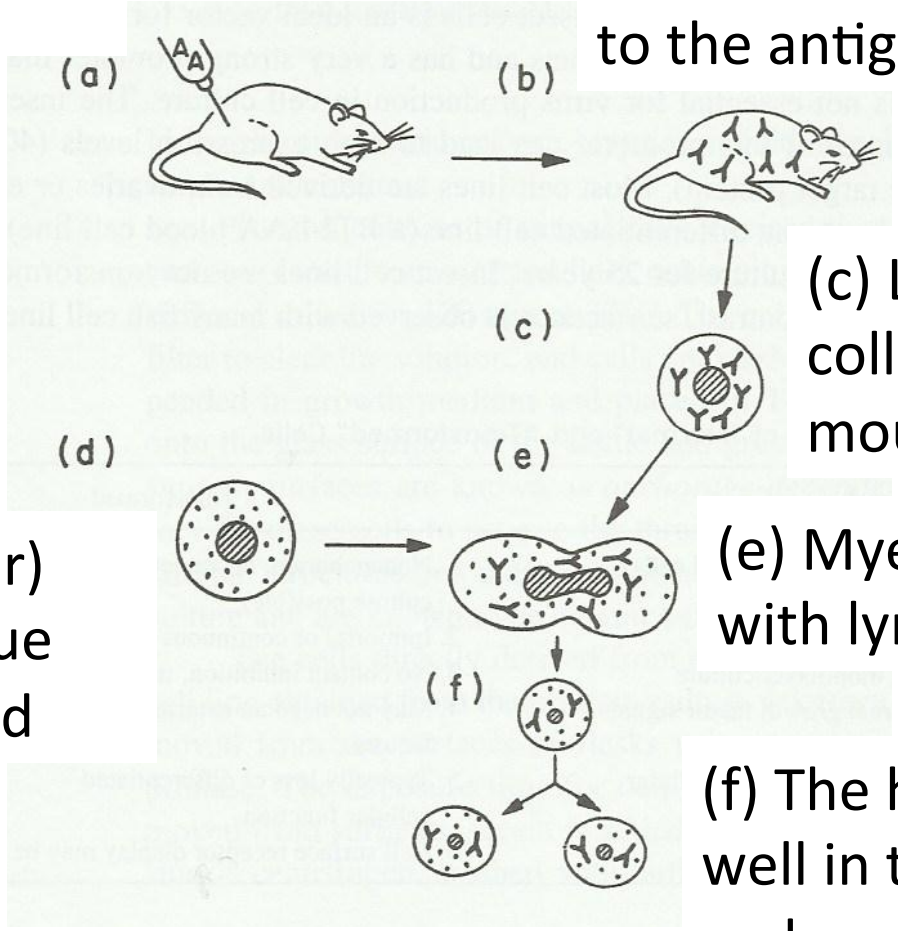
Hybridoma Cell

- Obtained by fusing lymphocytes (normal blood cells that make antibodies) with myeloma (cancer) cells
- Lymphocytes producing antibodies grow slowly and are mortal
- After fusion with myeloma cells, hybridomas become immortal, can reproduce and produce antibodies.

Steps in formation of a hybridoma for making antibody

(a) Antigen is injected into a mouse

(b) Lymphocytes in the mouse are activated to produce specific antibodies to the antigen



(c) Lymphocytes are collected from the mouse

(d) Myeloma (cancer) cells growing in tissue culture are produced

(e) Myeloma are fused with lymphocytes

(f) The hybrid cell grows well in tissue culture and makes a single monoclonal antibody

Serum

- A typical growth medium for mammalian cells contains serum (5-20%), inorganic salts, carbon and energy sources, vitamins, trace elements, growth factor and buffer in water.

- Serum is a cell-free liquid recovered from blood (FBS-fetal bovine serum; CS-calf serum; HS-horse serum)

- Serum is known to contain amino acids, growth factors, vitamins, certain protein, hormones, lipids and minerals.

Serum's function:

1.To stimulate cell growth and other cell activities by hormone and growth factors

2.To enhance cell attachment by certain proteins such as collagen and fibronectin

3.To provide transport proteins carrying hormones, minerals and lipids

Kinetic Growth of Mammalian Cell Culture

No.	Cells	Growth condition
1.	Mammalian cells	37°C, pH ~7.3 Doubling time: 12 – 20 h Need to be gently aerated and agitated Buffer used: Carbonate buffer/ CO_2-enriched air/HEPES
2.	Insect cells	28°C, pH 6.2
3.	Fish cells	25°C-35°C, pH 7 – 7.5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Stem Cells

Introduction

- Stem cells are cells characterized by their ability to differentiate into multiple cell types
- Different classes of stem cell have different levels of potential
- Stem cells are found in nearly all multi-cellular organisms, even us!
- Stem cell research expanded in the 1960s thanks to Canadian scientists Ernest A. McCulloch and James E. Till
  - Canada's greatest contribution to knowledge besides Ice Hockey

Stem Cell Requirements

- In order to be considered a stem cell, 2 requirements must be met:
  - **Self-renewal:** Stem cells must be able to go through multiple cell division cycles while remaining undifferentiated.
  - **Potency:** Stem cells must have the ability to differentiate into specialized cell types. This most often refers to the ability to differentiate into all or most cell types of the body, but lesser levels of potency can qualify as well.

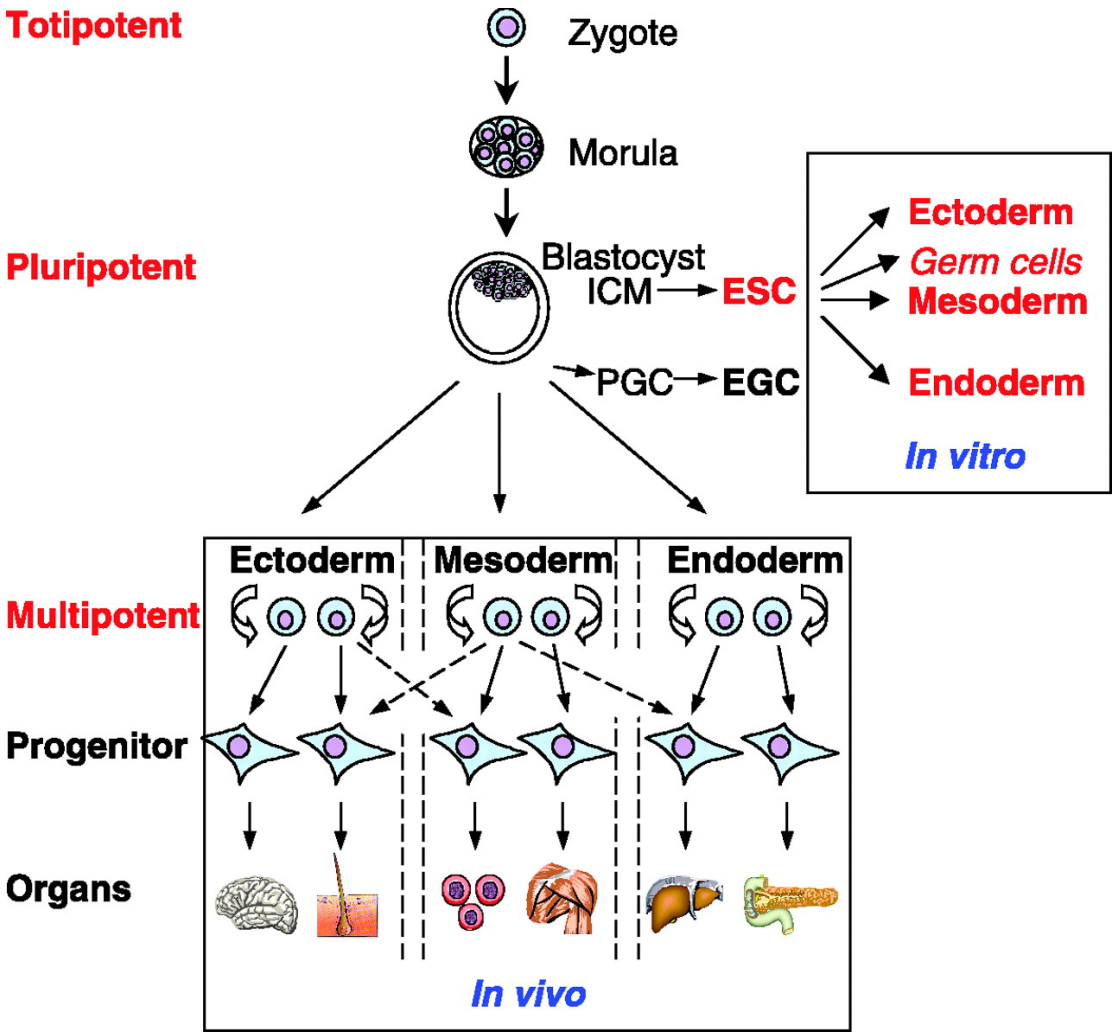
Potency Definitions

- **Totipotent (Omnipotent):** Stem cells that can differentiate into an entire organism. Results from fusion of egg and sperm.
- **Pluripotent:** Stem cells that can differentiate into any tissue type except for placenta tissue.
- **Multipotent:** Stem cells that can differentiate into multiple cells in a closely related family of cells.
- **Oligopotent:** Stem cells that can differentiate into only a few cell types (example: lymphoid stem cells).
- **Unipotent:** Stem cells that can differentiate into only one cell type but still possess self-renewal (example: muscle stem cells).

Other Definitions

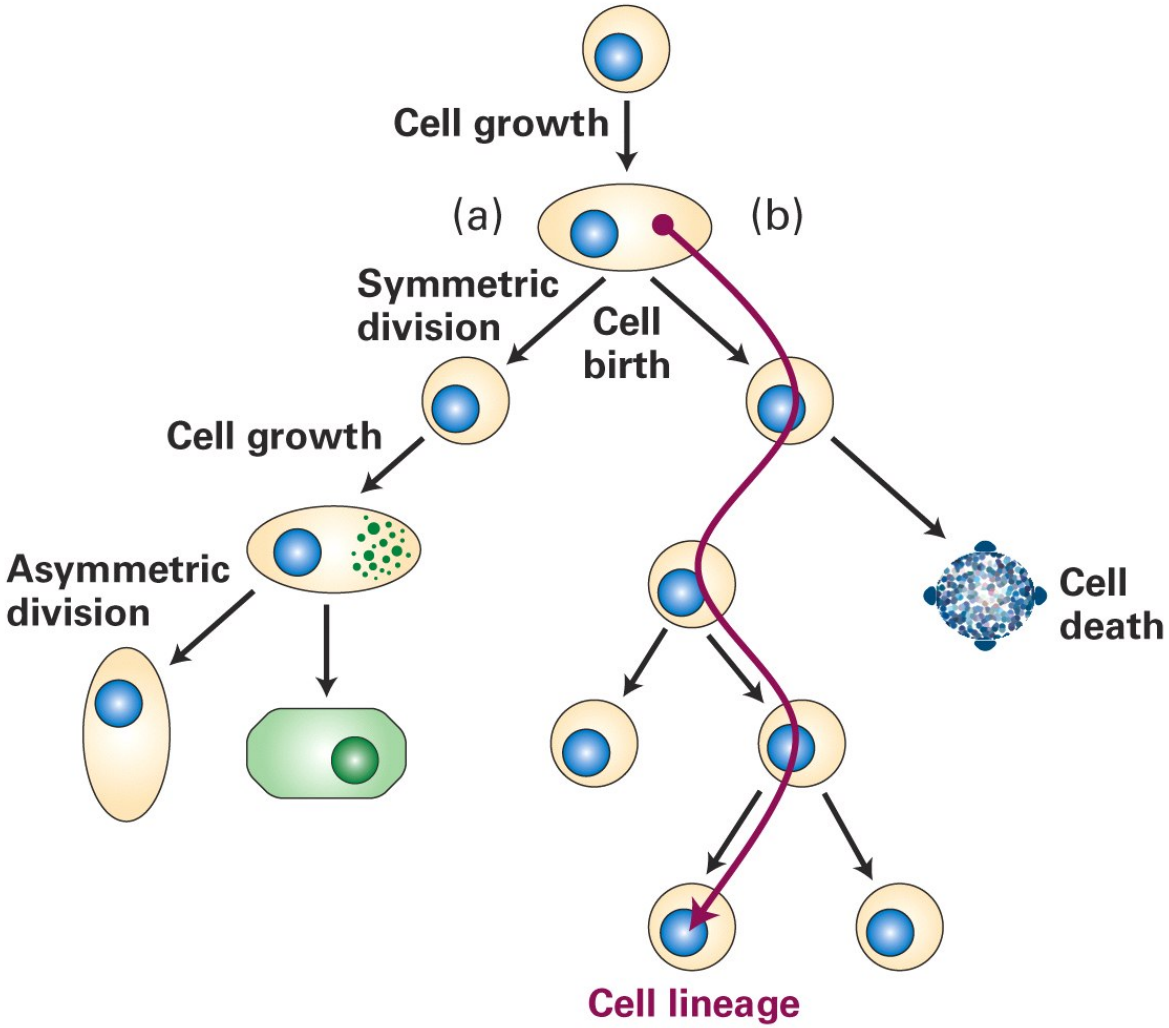
- **Progenitor Cell:** Multipotent or unipotent cell whose self-renewal can be limited. Differentiates into a specialized cell.
- **Differentiated Cell:** A cell at the end of a cell line that has become specialized for a particular function. Derived from progenitor cells.
- **Cell Lineage:** A series of cell divisions leading to specialized and differentiated cells. Similar to a family tree for cells.

Potency Hierarchy



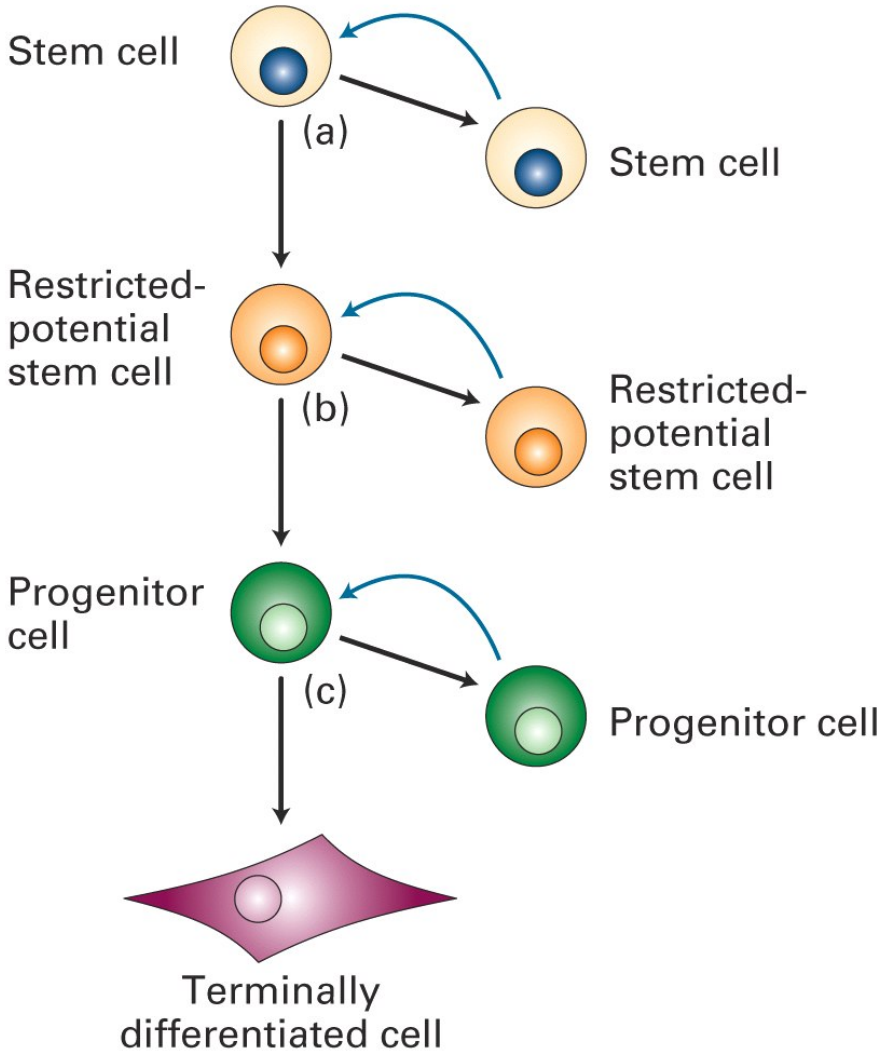
Wobus, A.M. et al. (2005)

Cell Lineage



Wang, N. (2010)

Stem Cell Lineage



Wang, N. (2010)

Types of Stem Cells

- **Embryonic:** Stem cells derived from the inner cell mass of a blastocyst
- **Fetal:** Primitive stem cells found in the organs of fetuses
- **Adult:** Stem cells found in developed organisms that can divide to form more differentiated cells
- **Amniotic:** Multipotent stem cells found in amniotic fluid
- **Induced pluripotent:** Cells reprogrammed through genetic engineering to become stem cells

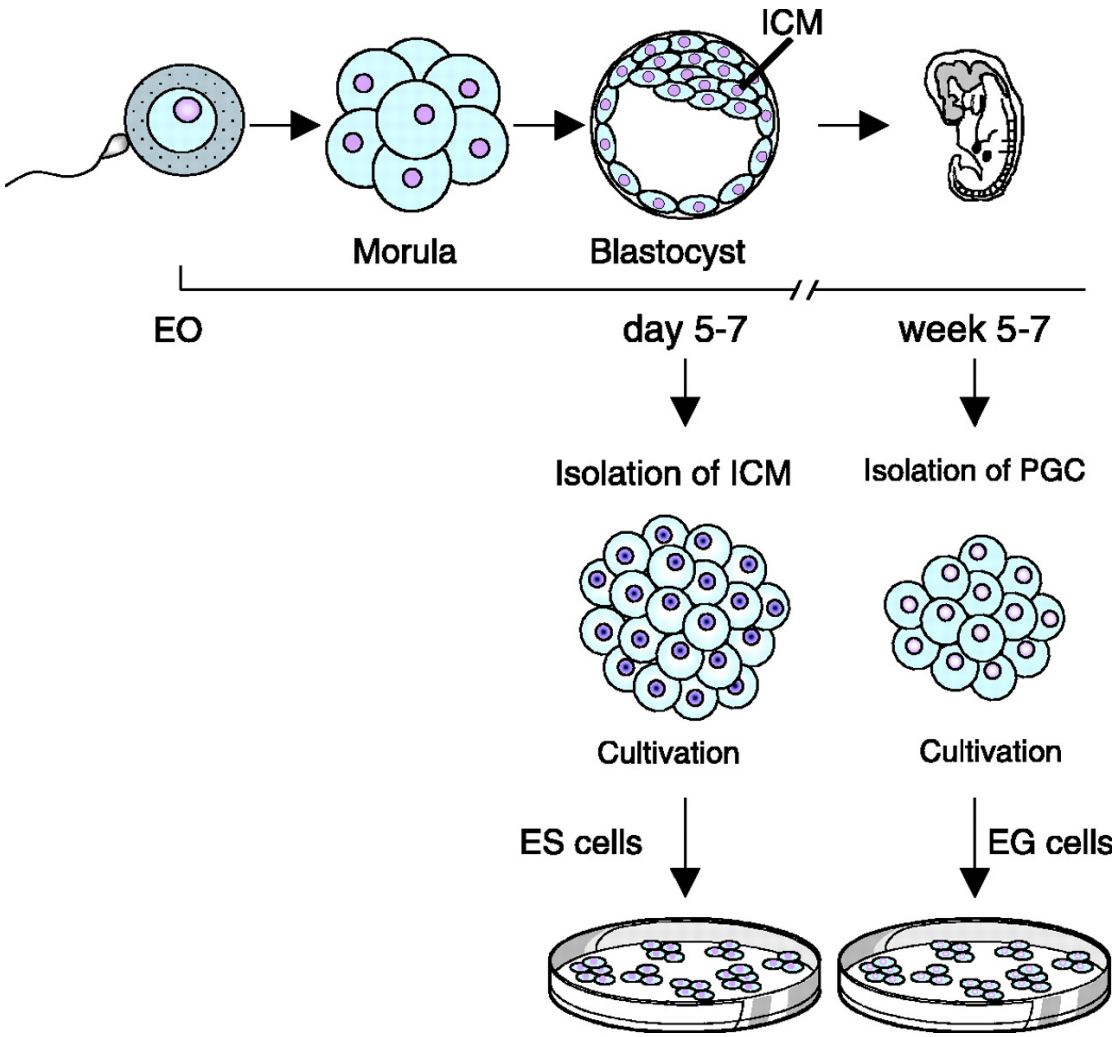
Adult Stem Cells

- Stem cells found in certain tissues that have the ability to divide into specific cell types
- Found in children and umbilical cord blood too!
- No need to destroy an embryo; avoids controversy
- Typically multipotent and restricted to certain cell lineages
- Have been used successfully for treatments for a long time via bone marrow transplants
- Examples: Mesenchymal, endothelial

Embryonic Stem Cells

- Derived from epiblast tissue of inner cell mass or early morula stage embryos
- Pluripotent cells that can give rise to any cell type of the three primary germ layers
  - Thus, any cell type in the body
- Require disruption of a growing embryo… controversy!
- Most research thus far has involved mice or human embryonic stem cells

Embryonic Stem Cell Collection



Wobus, A.M. et al. (2005)

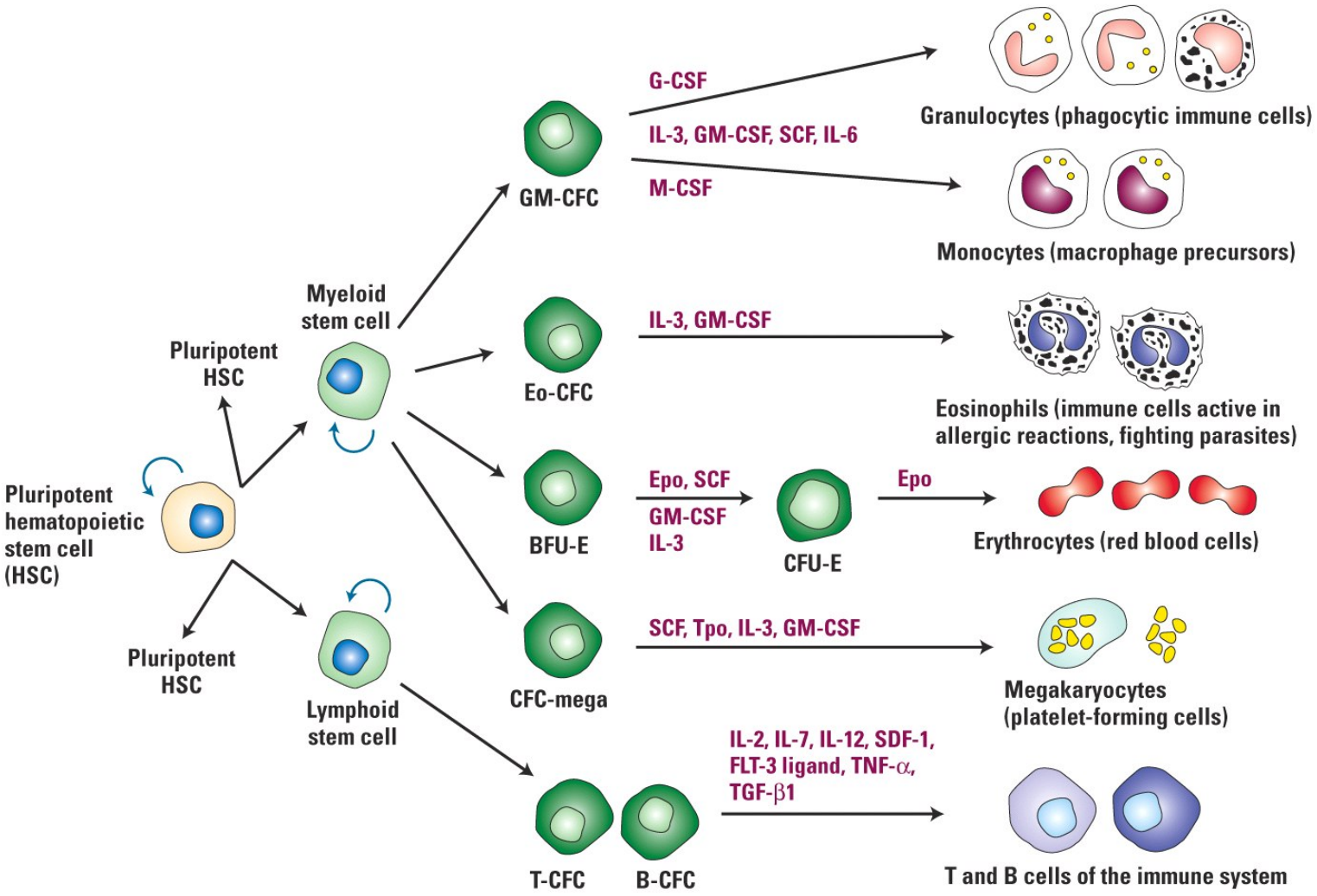
Embryonic Stem Cell Potential

- Pluripotent!!! Can differentiate into any cell type of a developed organism
- Can be used to replace "broken" or missing cell types resulting from certain diseases

Stem Cell Differentiation

- Several factors influence differentiation of stem cells, many of which are still not well understood
- Chemical factors: Presence of certain proteins and other macromolecules
- Physiological factors: Temperature, pH, oxygen levels, etc…
- Mechanical factors: Extracellular matrix stiffness

Stem Cell Differentiation

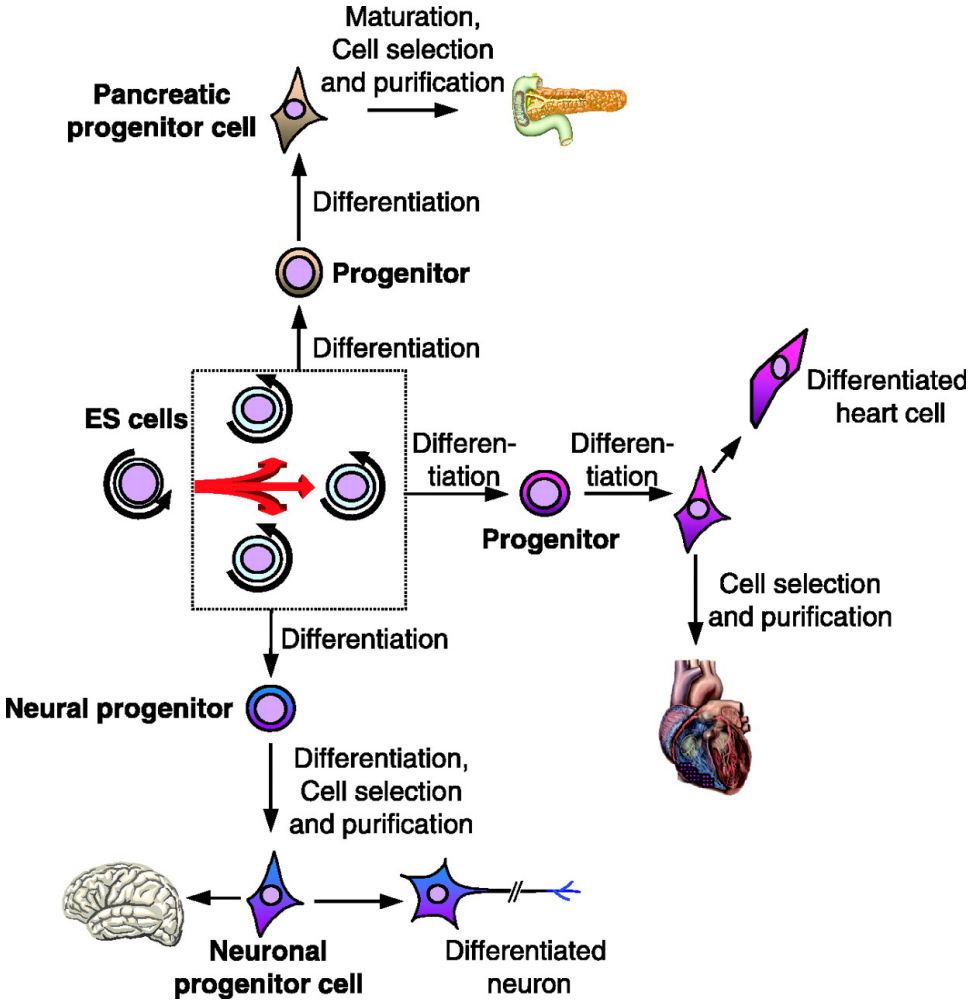


Wang, N. (2010)

Embryonic Stem Cell Therapy

- Advantages of embryonic stem cells over adult stem cells for therapeutic purposes:
  - Can be grown indefinitely in culture
  - Can be genetically manipulated
  - Numerous differentiation protocols have been established
- Numerous animal models show the potential of embryonic stem cell therapy
- Cardiac repair, vascular structure formation, neurorepair, diabetes treatment

Embryonic Stem Cell Therapy

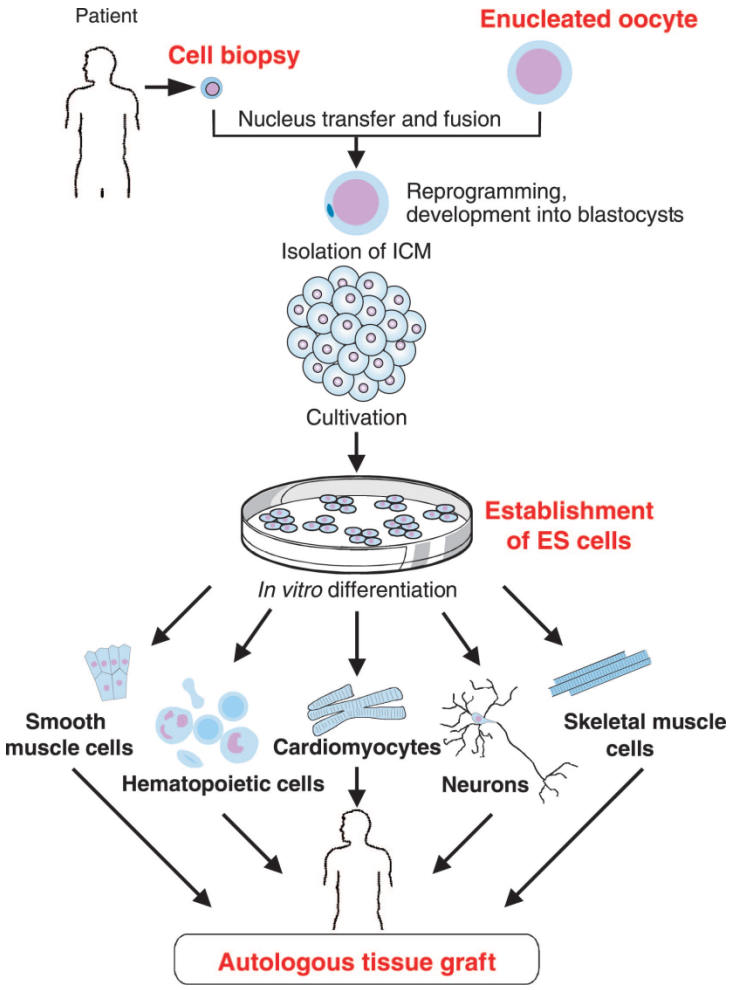


Wobus, A.M. et al. (2005)

Therapeutic Cloning

- Utilizes nuclear transfer techniques to produce pluripotent embryonic stem cells with the same genome as the nucleus of origin
- If these cells are transferred to a female uterus, reproductive cloning occurs – Dolly the sheep!
- If these cells are left to culture, they develop into a blastocyst
  - Embryonic stem cells can then be derived from the inner cell mass as per usual process
  - These cells avoid any risk of immunity or incompatibility with the host

Therapeutic Cloning

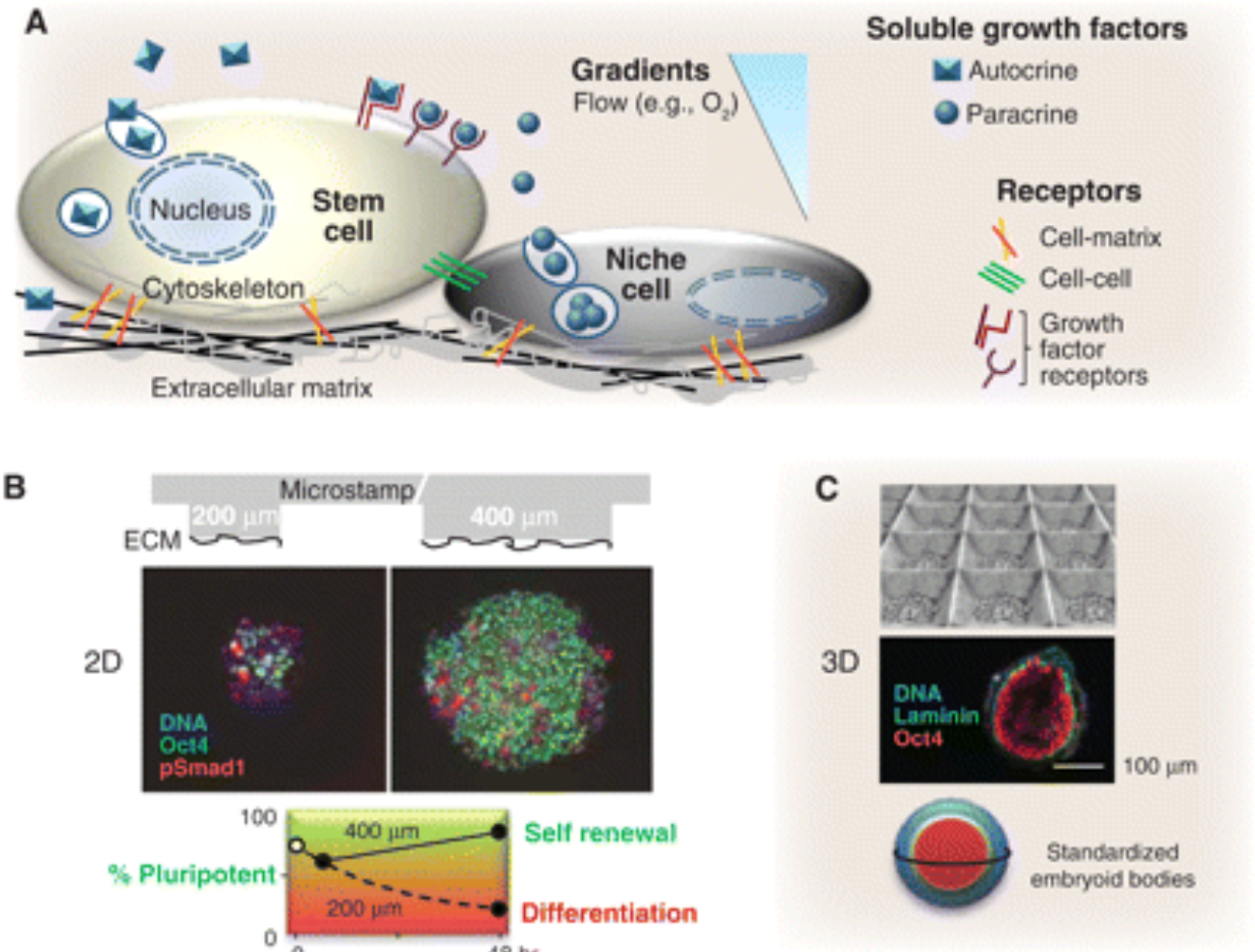


Wobus, A.M. et al. (2005)

Factors for Stem Cell Survival

- Stem cell survival and differentiation is influenced by a number of factors that must be controlled to ensure stem cell proliferation
- Stem cells require coordinated interaction with soluble factors, other cells, and extracellular matrices
- Specific soluble growth factors and cellular receptors are necessary for survival
- Certain extracellular environments also important for survival and specialization

Factors for Stem Cell Survival



Discher, D.E. et al. (2009)

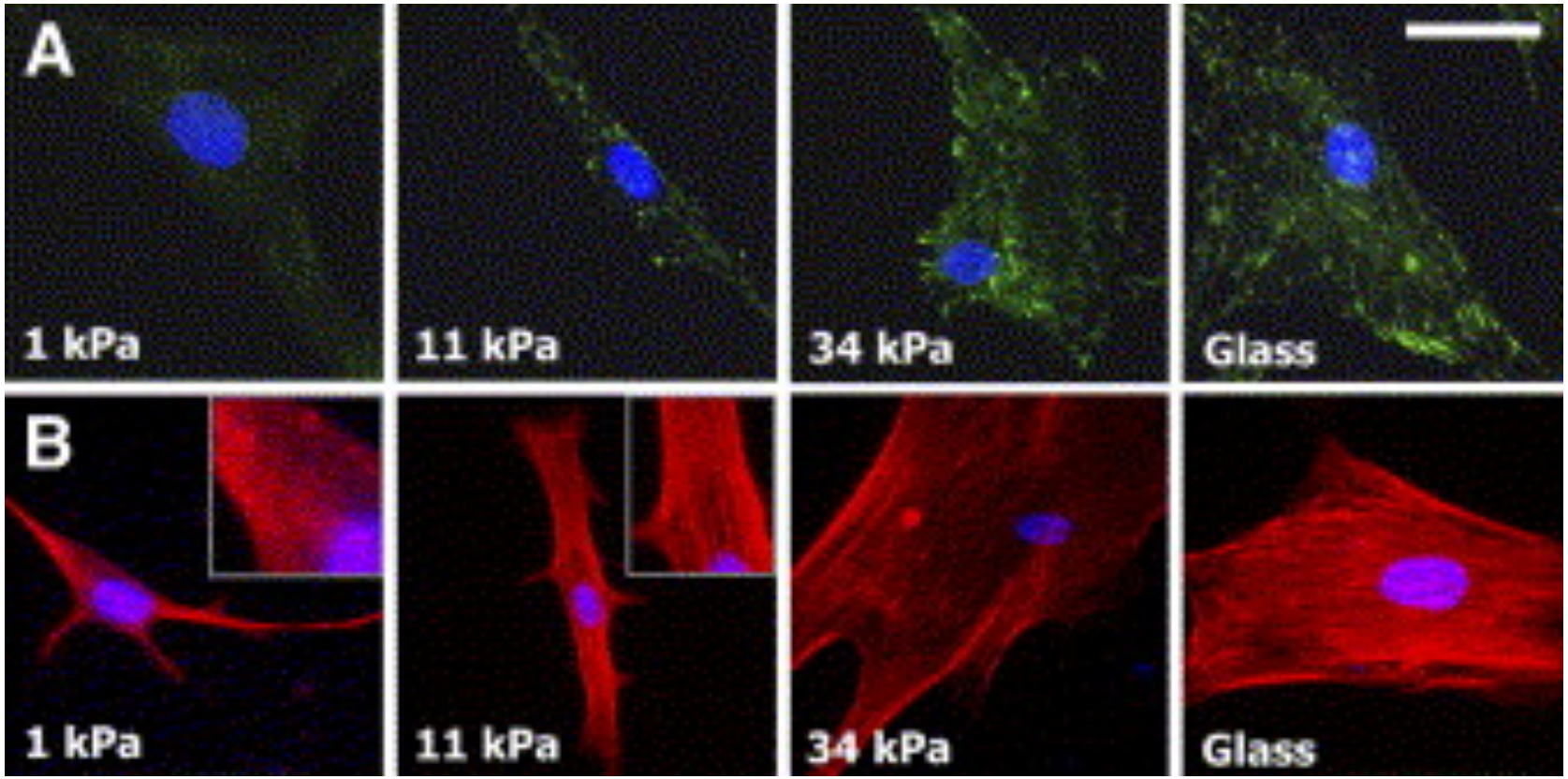
Substrate Stiffness Effects

- Stem cells are strongly influenced by extracellular substrate stiffness
- As stem cells differentiate, their stiffness and elasticity is proportional to substrate stiffness
- Cells react to operate in their specific environment
- Extracellular substrate ultimately affects specific cell properties
  - Presence of adhesion proteins on cell surface
  - Presence of structural proteins within the cell

Substrate Stiffness Effects

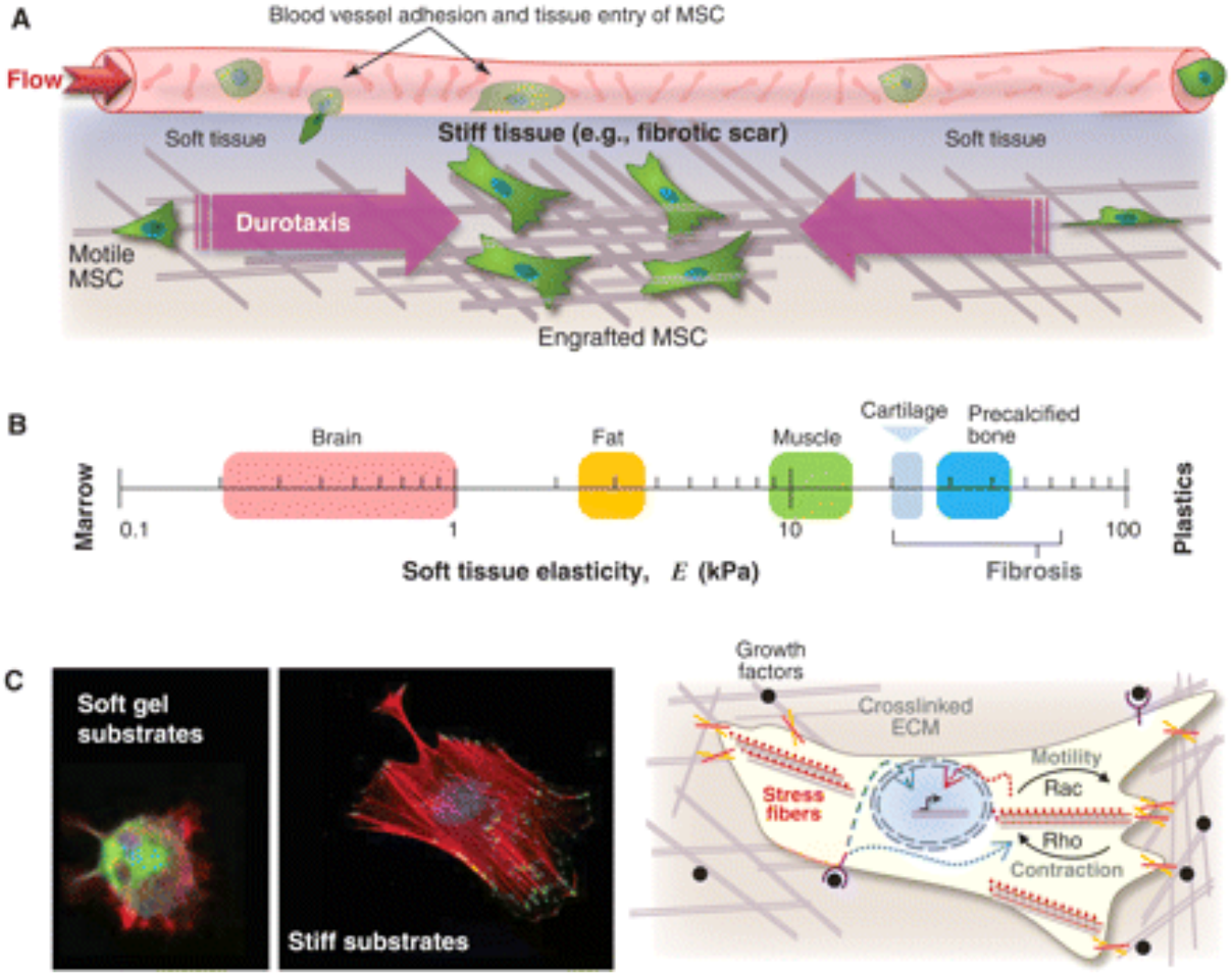
Adhesion Proteins

Actin



Engler, A.J. et al. (2006)

Substrate Stiffness Effects



Discher, D.E. et al. (2009)

Stem Cells and Understanding Cancer

- Embryonic stem cells share many features that cancer cells possess
  - Unlimited proliferative capacity
  - Clonal propagation
  - Lack of both contact inhibition and anchorage dependence
- Embryonic stem cells commonly lead to teratomas or teratocarcinomas when transplanted to extrauterine sites
- Better understanding of stem cells may lead to better understanding of cancer