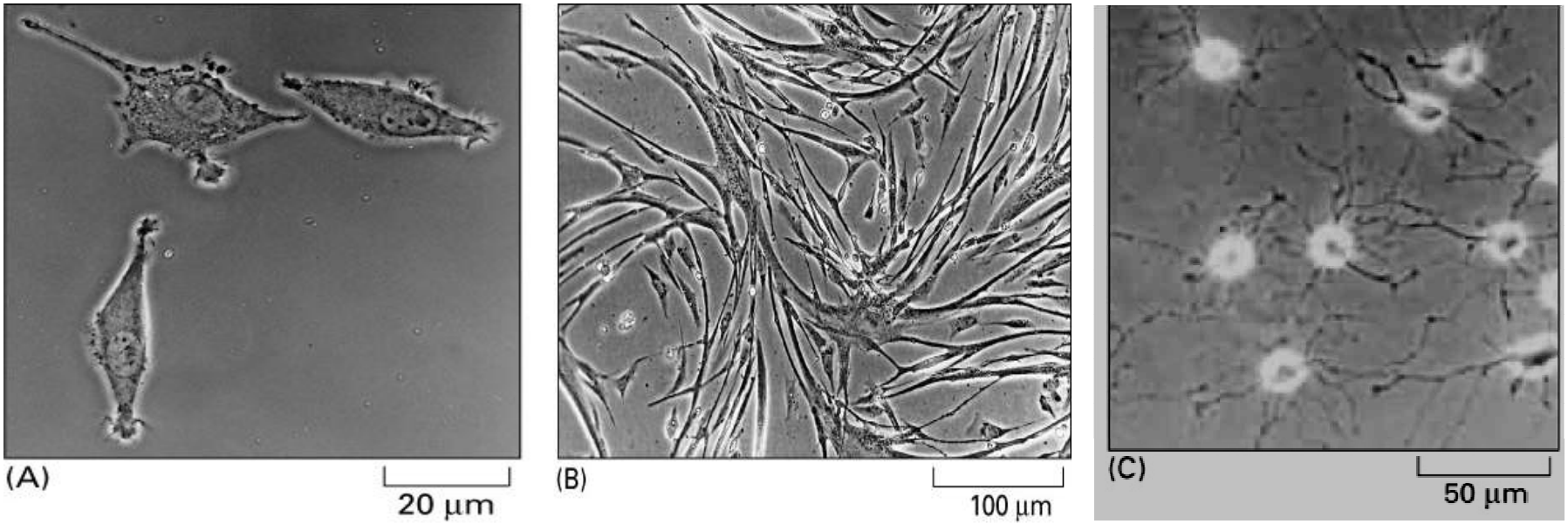
Chapter 12

Animal Cell Cultures

Cells in culture



(A) Fibroblasts in culture

(B) Myoblasts in culture shows cells fusing to form multinucleate muscle cells

(C) Oligodendrocyte precursor cells in culture

What is cell culture used for?

- **Model systems for**

  Studying basic cell biology, effects of drugs on cells, and aging & nutritional studies

- **Toxicity testing**

  Study the effects of new drugs

- **Cancer research**

  Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells

- **Vaccine production**
- **Genetic Engineering : Production of commercial proteins**
- **Stem cell therapy**
- **Gene therapy**

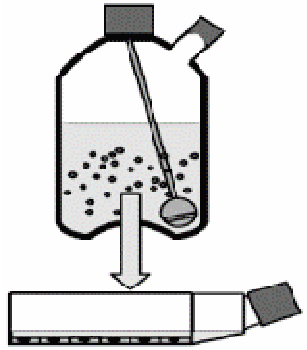
  Cells having a functional gene can be replaced to cells which are having non-functional gene

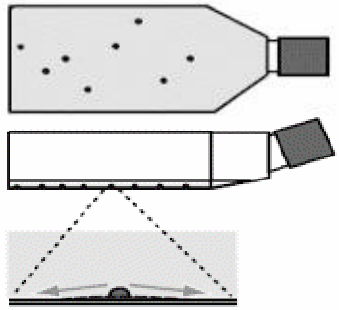
Advantages of working with cultured cells over intact organisms

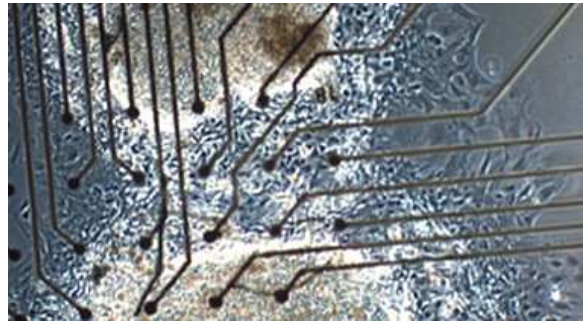
- More homogeneous than cells in tissues
- Can control experimental conditions
- Can isolate single cells to grow into a colony of genetically homogeneous clone cells (cell cloning, clone)

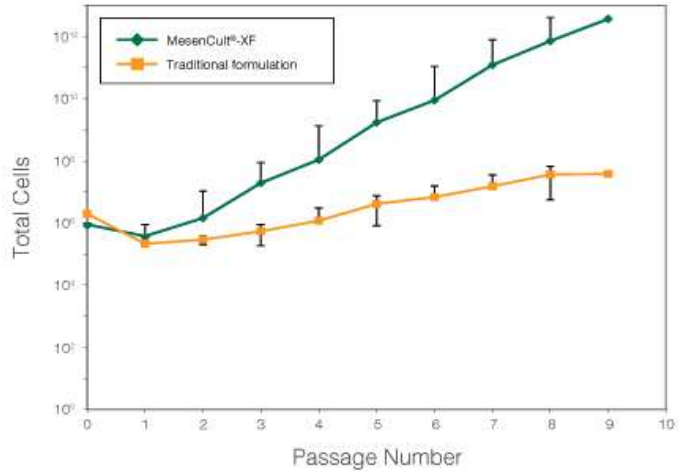
Establishing cells in culture: primary cells

- The dissociation of the tissue fragments into its component cells by treatment with proteolytic enzyme (trypsin).
- The cells multiply at a constant rate over successive transfers and such cells comprise cell strain.
- Cell strains divide only a finite number of times before their growth rate declines and they die.
- Human cells generally divide only 50-100 times before dying.

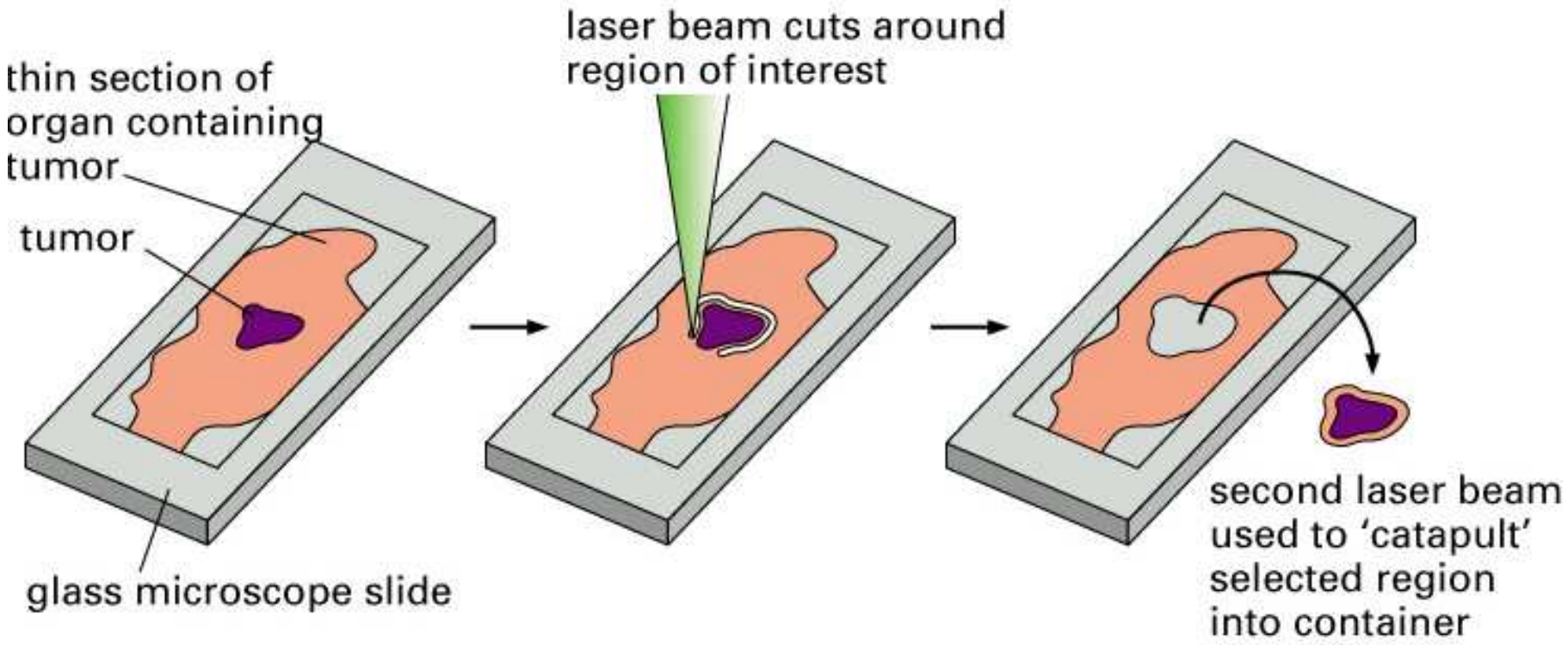






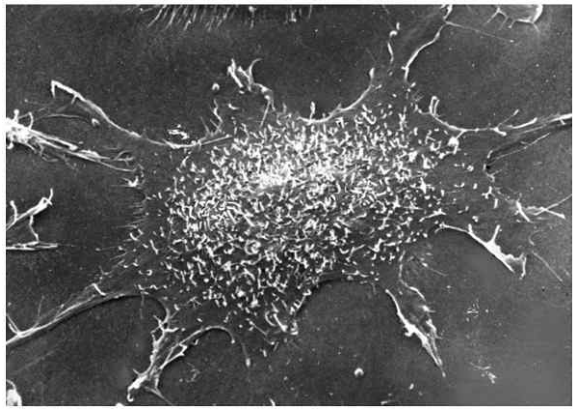


Microdissection techniques allow selected cells to be isolated from tissue slices

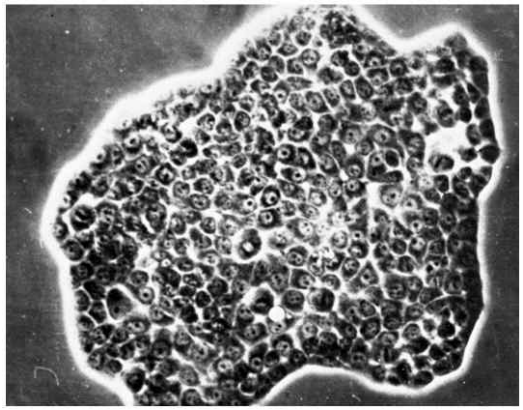


Growth of animal cells in culture

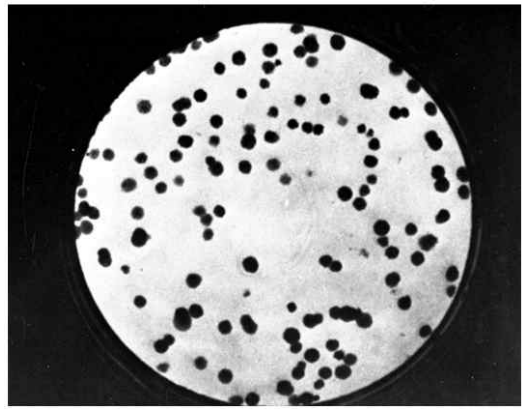
- Requires rich media including essential amino acids, vitamins, salts, glucose, and serum
- Most grow only on special solid surfaces



A single mouse cell



A colony of human HeLa cells



Many colonies in a petri dish

Components of media for animal cell culture

- **Serum (5-10%, from fetal calf or bovine)**
- **Balanced salt solution**
  - Essential inorganic ions
  - The correct osmolarity
  - The correct pH
- **Glucose (5–10 mM)**
- **Amino acids**
- **Vitamins**
- **Buffer : sodium bicarbonate or HEPES**
- **Phenol red : a pH indicator dye**
- **Antibiotics**
  - Penicillin, streptomycin : to prevent bacteria growth

Preparation of media and equipment

- Purity of water (distilled, deionized water)
- Media sterilization
  - Filtration : heat-labile growth medium
- The culture containers are kept in an incubator at 37^{o}C in an atmosphere of 5% CO_2 and 95% air.

Most cultured animal cells grow only on solid surfaces

- **Interaction between cells & cell and extracellular matrix**
- **ECM (extracellular matrix)**
  - Roles: binding the cells in tissue together
  - Common components: collagen, hyaluronic acid (proteoglycan or glycoprotein)
  - The exact matrix compositions vary depending on tissue type
- **Most cell types adhere to grow on specially treated plastics with negatively charged groups on surface**
- **Some tumors cells can be grown in suspensions**

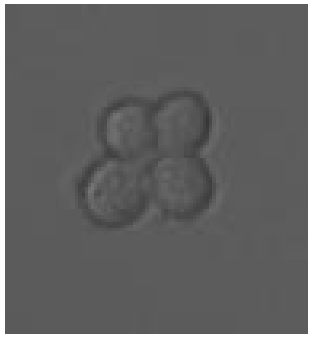
Primary cells and cell lines

- **Primary cell** cultures are established from animal tissues
- Most primary cells isolated from an animal tissues grow and divide for a limited period of time (about 50 doublings), then eventually die
- Certain “transformed cells” may arise, which are immortal and can be used to form a **cell line**
- Transformed cells may be derived from tumors or may arise spontaneously

Modes of cell growth

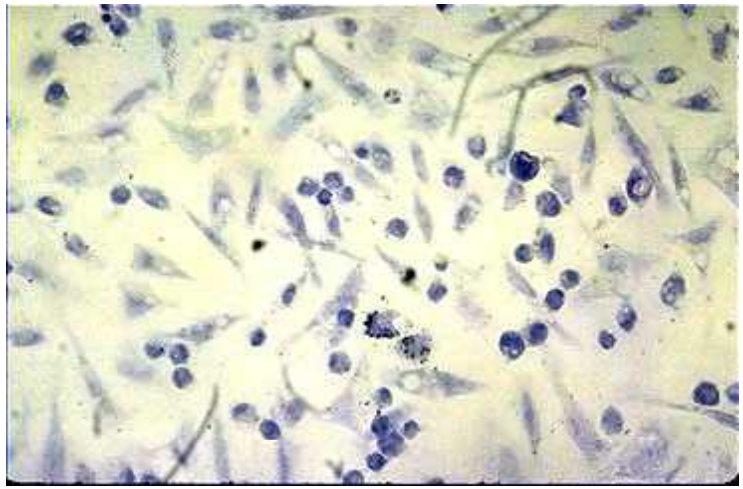
- **Anchorage-dependent cells**
  - Cells that attach to surfaces for survival and growth
  - most cell types

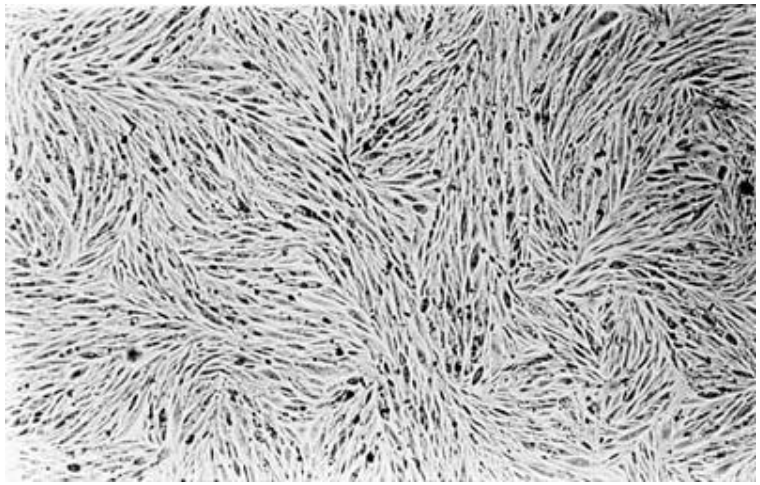
- **Suspension cells ; anchorage-independent cells, transformed cells, some blood cells**
  - Adhere poorly, and are held in suspension



Confluency

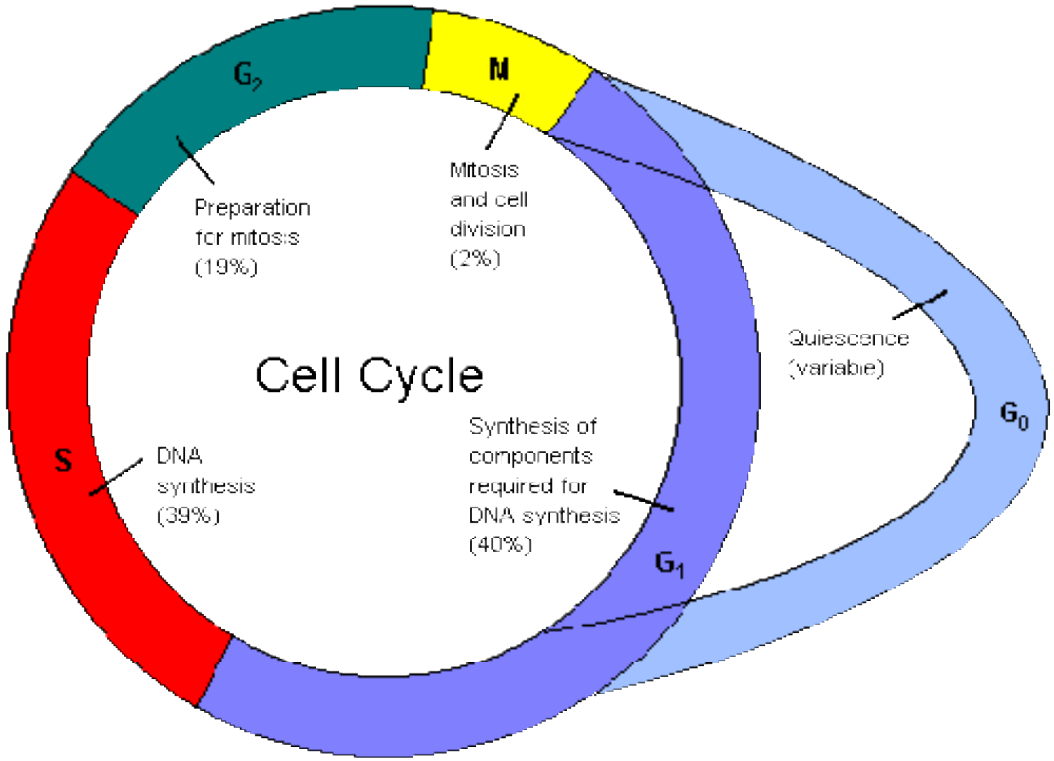
- **How "covered" the growing surface appears**
- **This is usually a guess**
- **Optimal confluency for moving cells to a new dish is 70-80%**
  - too low : cells will be in lag phase and won't proliferate
  - Too high : cells may stop growing and undergo unfavorable changes





Contact Inhibition

- When cells contact each other, they cease their growth and arrest in G0 phase of the cell cycle
- Transformed cells will continue to proliferate and pile upon each other



Passage number

- The number of times the cells have been removed (or “split”) from the plate and re-plated.

Cell lines

TABLE 8–2 Some Commonly Used Cell Lines

CELL LINE*	CELL TYPE AND ORIGIN
3T3	fibroblast (mouse)
BHK21	fibroblast (Syrian hamster)
MDCK	epithelial cell (dog)
HeLa	epithelial cell (human)
PtK1	epithelial cell (rat kangaroo)
L6	myoblast (rat)
PC12	chromaffin cell (rat)
SP2	plasma cell (mouse)
COS	kidney (monkey)
293	kidney (human); transformed with adenovirus
CHO	ovary (chinese hamster)
DT40	lymphoma cell for efficient targeted recombination (chick)
R1	embryonic stem cells (mouse)
E14.1	embryonic stem cells (mouse)
H1, H9	embryonic stem cells (human)
S2	macrophage-like cells *(Drosophila)*
BY2	undifferentiated meristematic cells (tobacco)

*Many of these cell lines were derived from tumors. All of them are capable of indefinite replication in culture and express at least some of the special characteristics of their cell of origin. BHK21 cells, HeLa cells, and SP2 cells are capable of efficient growth in suspension; most of the other cell lines require a solid culture substratum in order to multiply.

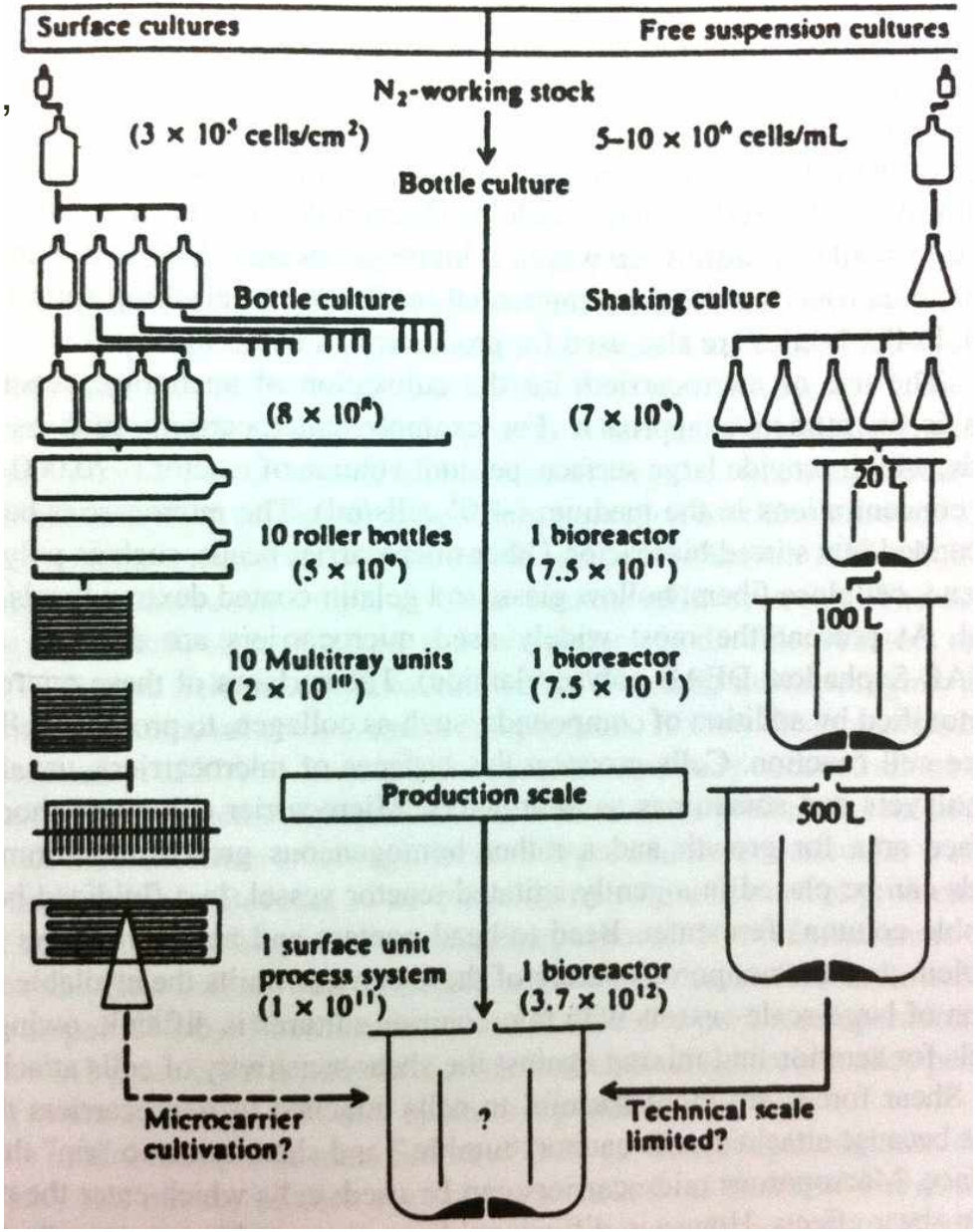
Scale-up of animal cell culture

Tissue culture dishes, T-flasks

Roller bottles

Multi-tray

Microcarrier culture



Flask shaking culture

Spinner flasks

Stirred tank reactor

Cell culture containers

- Tissue culture dishes, T-flasks

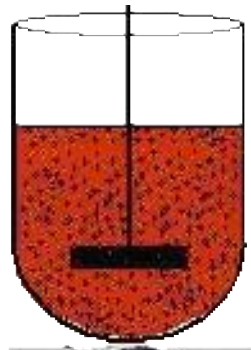
- Spinner flasks

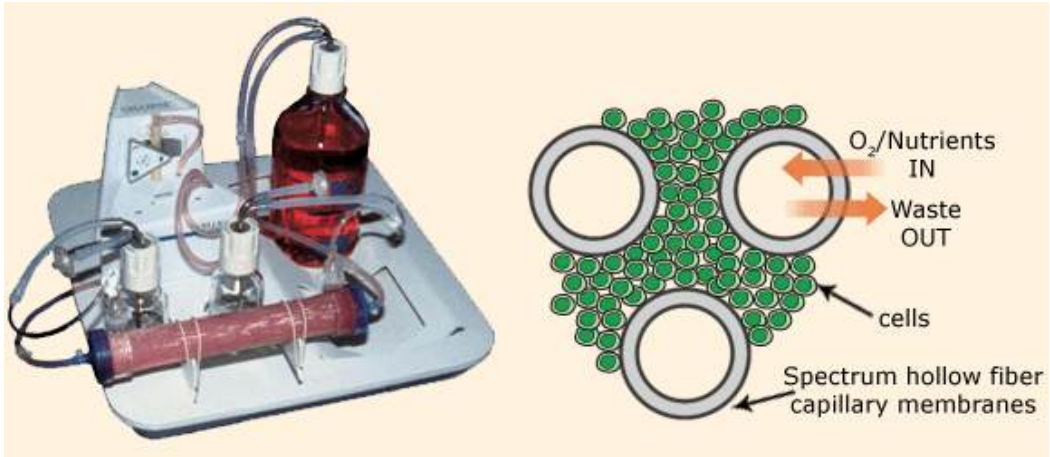
- Multi tray

Large-scale cell culture

- **Suspension cells**
  - Stirred tank reactor
  - Airlift fermentor

- **Anchorage-dependent cells**
  - Roller bottles
  - Microcarrier beads:
    - dextran or synthetic polymer, porous or solid microcarrier
    - porous matrix protects cell against shear in agitated cultures
  - Hollow-fiber apparatus
    - air and carbon dioxide are passed through the lumen of fibers

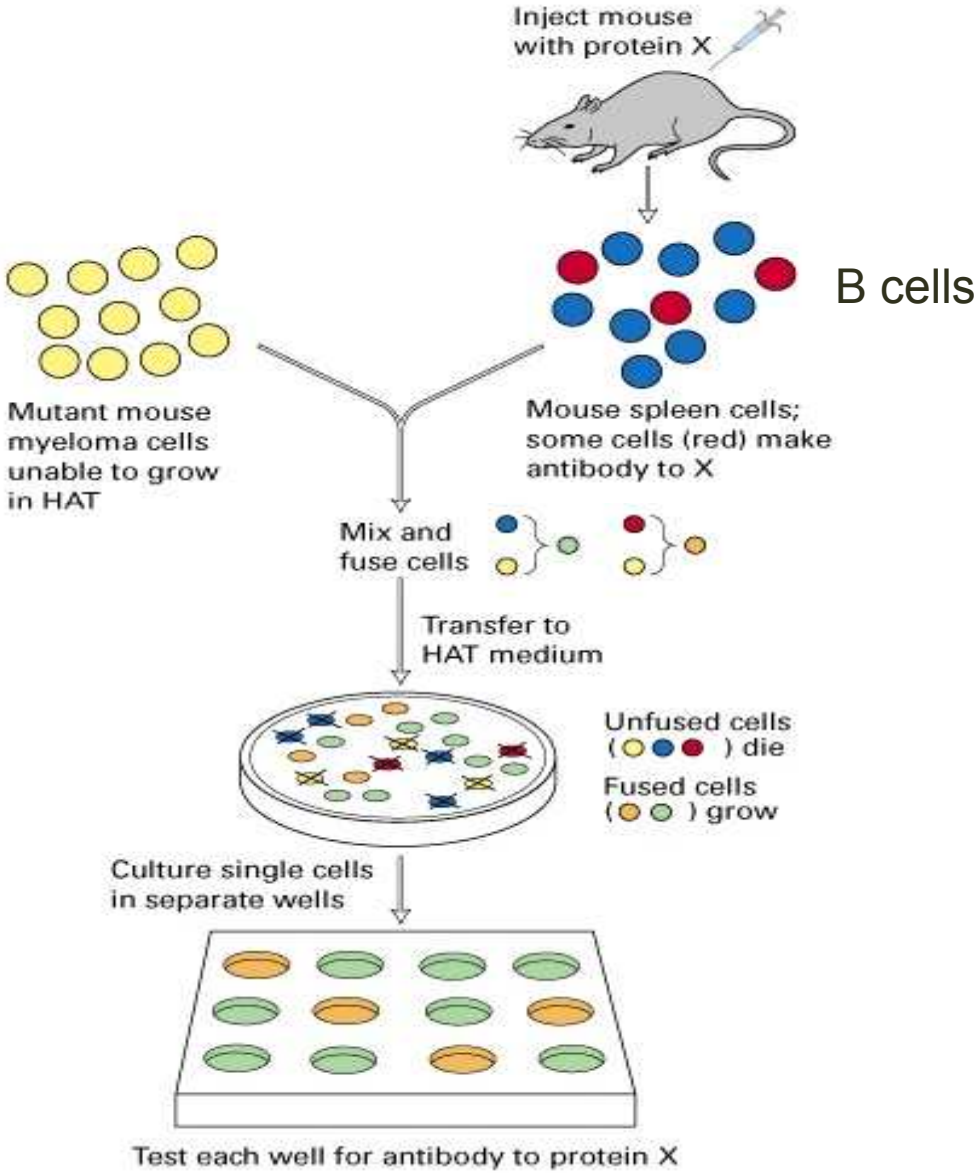




Products of animal cell culture

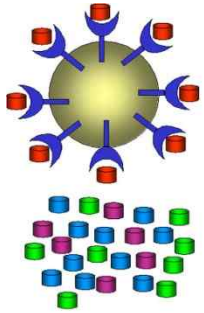
- **Monoclonal antibodies**
- **Immunobiological regulators**
  - Interleukines
  - Interferon
  - EPO
- **Virus vaccines**
- **Hormones**
- **Enzymes**
- **Whole cells**
  - stem cells, primary cells

Producing a monoclonal antibody to protein X



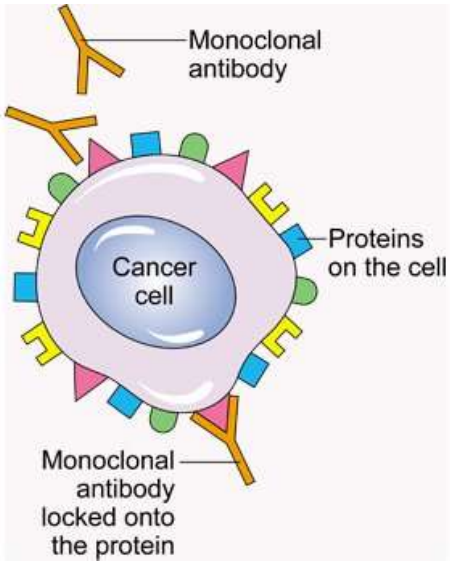
Applications of monoclonal antibody (MAb)

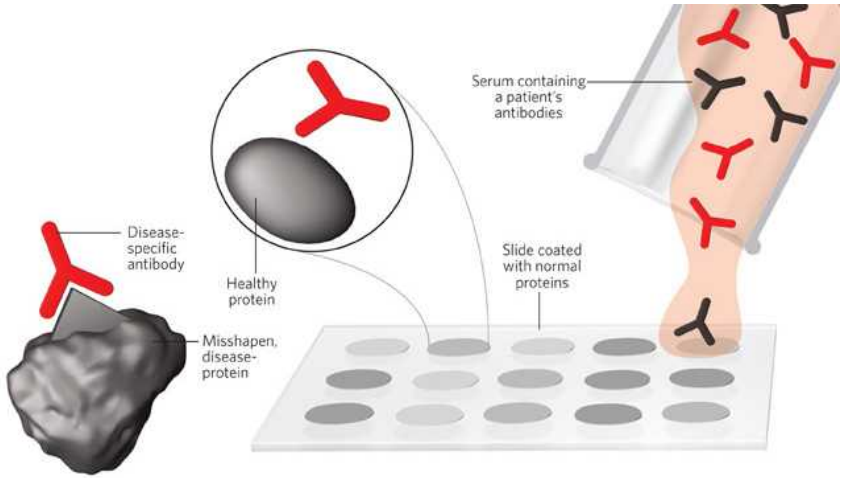
- **Biological separation**
  - Affinity chromatography



- **Diagnosis**
  - To detect drug, toxin, and other biological compounds

- **Cancer treatment**
  - Targeting to cancer molecules





Immunobiological regulators

- **Interleukines**
  - Anticancer drug
- **Interferon**
  - A class of proteins that are produced by white blood cells as part of the body's natural immune response as soon as the body is exposed to attack by viruses or tumor cells
  - Antiviral action
  - Anticancer drug

Virus vaccines

- Induce protective immune response upon injection
- Virus vaccines are produced by propagation in animal cells
- “killed” virus : inactivated
- “attenuated” virus : weakened virus

Hormones : Erythropoietin (EPO)

- **Glycoprotein (36kDa, 165 A.A.)**
- **Helping the production of RBC**
- **Treatment of anemia associated with cancer/chemotherapy**

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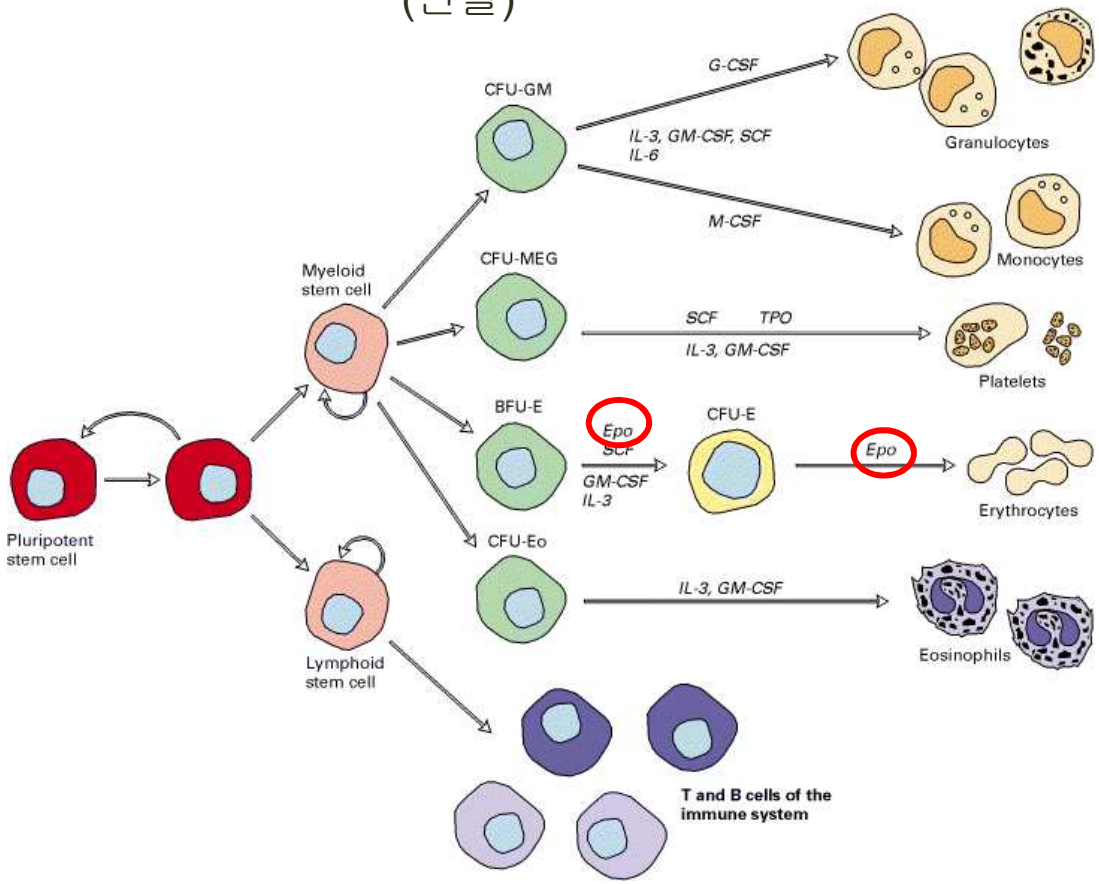

Formation of differentiated blood cells from hematopoietic stem cells in the bone marrow

Enzymes

- Urokinase : a compound dissolving blood clotting
- Trypsin, pepsin, collagenase

Values of animal cell products

protein	Annual sales M$	product name	company	cells	fist year licensed	extent of licence
tPA	200.00	activase/actilyse	Genentech	CHO	1987	broad
Erythropoietin	1,962.00	Epogen/Eprex/Procrit	Amgen	CHO	1989	broad
Erythropoietin	2,709.00	Procrit	Johnson & Johnson	CHO	1990	Japan, Europe
Human Growth Hormone	500.00	Suizen		C127	1989	broad
HepBsAg Vaccine	200.00	GenHevacB Pasteur		CHO	1989	France
Interferon-beta	761.00	Avonex	Biogen	CHO	1996	USA
Monoclonal antibody	200.00	OKT3		Hybridoma	1986	broad
Monoclonal antibody	418.00	Reopro	J&J Lilly	Hybridoma	1994	USA
Human insulin	1,115.00	Humulin	Eli Lilly	E.Coli	1982	USA
Monoclonal antibody	280.00	Herceptin	Genetech	Hybridoma	1998 (Nov)	USA
figrastin	1,224.00	Neupogen	Amgen			
anti-thrombin	377.00	Kogenate	Bayer			
human growth hormone	467.00	Gentropin	Genetech			
r-HepB surface Ag	251.00	Energix-B	Genetech GlaxSKB			
Interferon A	600.00	Intron A	Biogen/Schering P			