

Basic Cell Culture

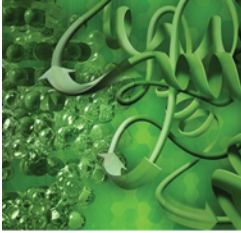
An Introduction to Growing and Maintaining Mammalian Cells in Culture





Introduction

- Established in the early 1900s
- Further developed during the 1940s and 1950s to support virology research and to provide a tool for producing virus for vaccines
- Used today for a wide variety of studies
 - Model systems
 - Understanding disease states
 - Parkinson's disease
 - Diabetes
 - Multiple sclerosis
 - Examining tissue and organ development



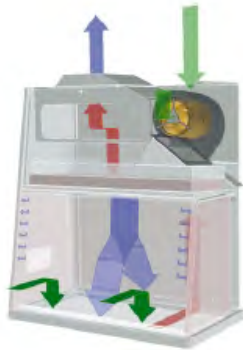
What you need . . . (in addition to normal lab equipment and supplies)

- Cells!
- Laminar flow hood (biological safety cabinet)
- CO₂ incubator (for most cells)
- Plastics for growing cells
- Sterilization filters (0.2 μm)
- Media
- Microscopes
- Cell counting tools — hemocytometer or instrument
- Media and supplements
- Other: pipette aid, aspiration pump, centrifuge, water bath, cold storage (refrigerator), cryopreservation equipment



Laminar Flow Hood

- Maintains sterile working area
- Horizontal or vertical configurations
- No hood necessary if you have an isolated “clean” room
- Vertical or biological safety cabinets
 - Usually equipped with UV light for sterilization of the work surface — used before and after (not during) work
- Hoods are not a storage area!



Arrows indicate airflow





Incubator

- All mammalian cells require a CO₂ incubator
- Most mammalian cells require
 - Temperature = 37°C
 - Humidity = 95%
 - CO₂ = 5%
 - May be water jacketed





Main Cell Types

- Cell lines, immortalized (or established)
 - The ATCC (American Type Culture Collection) and ECACC (European Collection of Cell Cultures) are the main sources; they contain over 3,400 cell lines from over 80 different species
 - Cells are stable, can be frozen (liquid nitrogen), and are grown for a defined number of passages
 - Examples of common cell lines: 3T3, 293, CHO, COS, HeLa
- Primary cells
 - Cultured directly from a tissue or organ
 - Most have a limited life span, and undergo senescence after a finite number of population doublings
 - Isolated from tissues for culture by several methods including
 - Purified from blood
 - Released from soft tissue by enzymes such as collagenase, trypsin, or pronase





Compare and Contrast

Cell Lines	Primary Cells
Widely used, but information gained may not be as biologically relevant as that from primary cells	More medically or physiologically relevant
Easier to grow; proliferate indefinitely	Sometimes difficult to grow; limited life span in culture
Can usually be frozen for future use	May not survive freezing
May be modified (e.g., many originate from cancerous tissue), resulting in loss of properties of parent	No modifications
Easy to obtain – purchase from ATCC or obtain from another lab	Obtained from animal or tissue
<i>Should</i> be genetically identical (care should be taken if obtained from a source other than ATCC, may not be a true cell line)	May be a mixed population

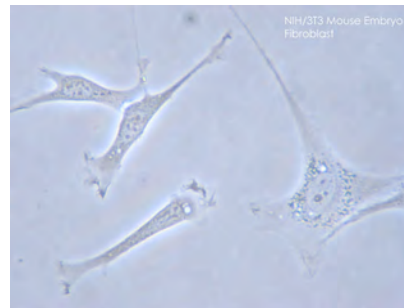


Common Cell Lines From Tissue

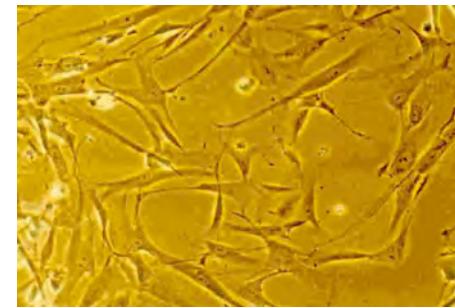
- Epithelial tissue (provides a covering): CHO (Chinese hamster ovary), HeLa, Hep-2, MCF-7, U373
- Connective tissue (e.g., fibroblast): 293, 3T3, COS
- Muscle tissue: vascular smooth muscle cells
- Nervous tissue: SKN
- *Many cell lines originate from cancerous tumors; these cells are grown attached to a surface and are known as ADHERENT cells*



CHO Cell
Reprint Flickr



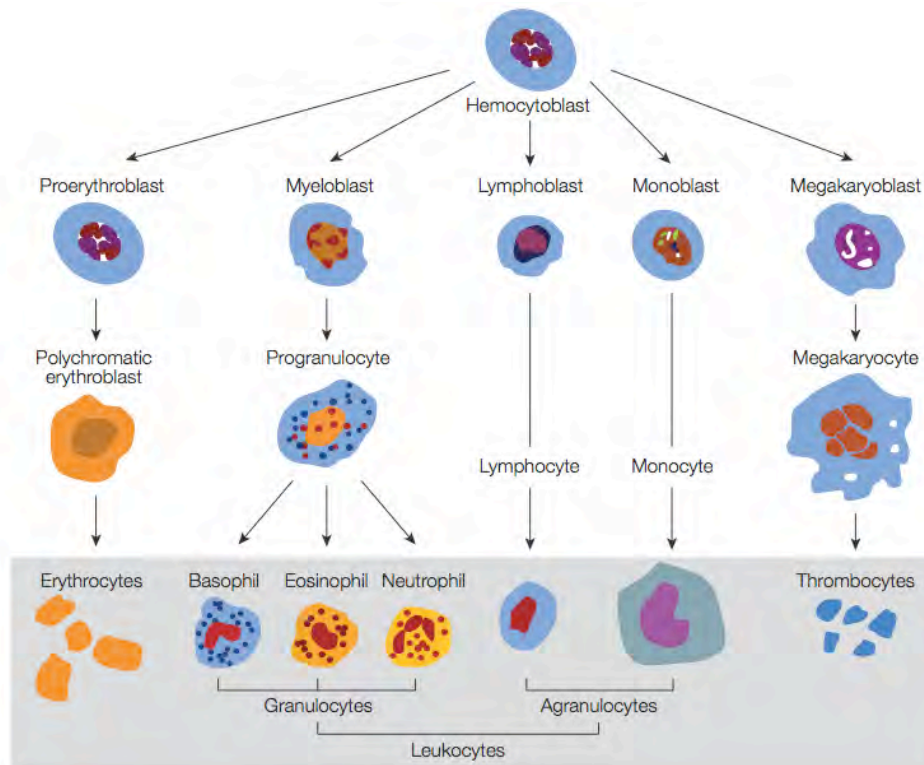
NIH/3T3 Mouse Embryo Fibroblast
Reprint Wiki



Smooth Cardiac Muscle
Reprint Wiki



Blood Cells



- These cells grow only in suspension; lymphocytes are commonly used
 - Lymphocyte or lymphoblast: HL-60, Jurkat



Common Media

- Most common media

- **D-MEM / F12 Media**

- Contains D-glucose (dextrose), L-glutamine, HEPES buffer, hypoxanthine monosodium salt, linoleic acid, lipoic acid, putrescine dihydrochloride, phenol red indicator, sodium pyruvate, thymidine, amino acids, vitamins

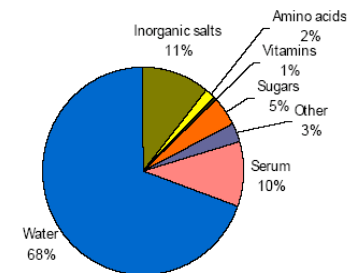
- **RPMI 1640 w/ Glutamine**

- Contains L-glutamine, HEPES buffer, phenol red indicator

- Common supplements

- HEPES buffer
 - EDTA
 - L-glutamine
 - Antibiotics: penicillin, gentamicin, Pen-Strep solution
 - Antifungals: Fungizole
 - Fetal calf/bovine serum

Cell Culture Medium



Various media, pH 7.2–7.5; most include a dye indicator to show changes in pH



Cell Culture Vessels

- Adherent* cells or suspension cells
* Grown attached to a surface



- T-flasks
(T-25, T-75, T-150; the number indicates cm² of surface area)



- Typical cell density: 5×10^4 – 1×10^7 /ml
- Vendors: Corning, Nunc, BD, Wheaton

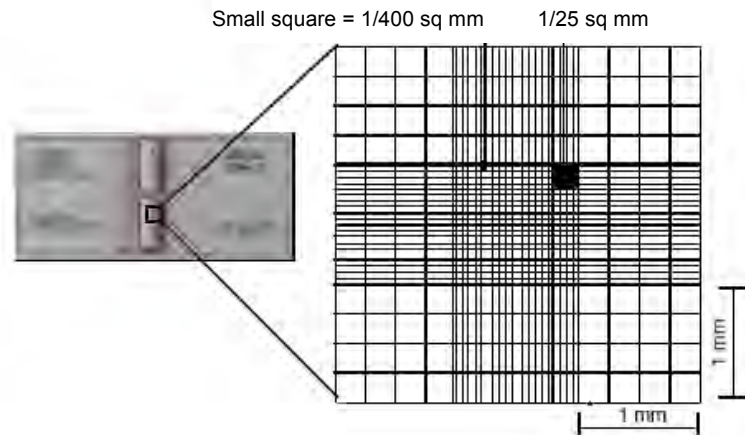
- Suspension cells only

- Spinner flasks





Counting Cells



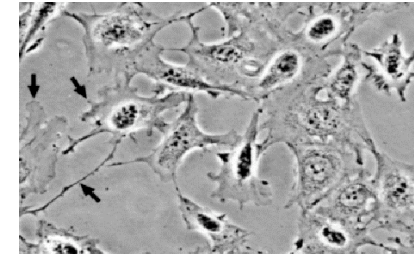
Cells are usually counted using hemocytometers (list price \$300). A small aliquot of the cell suspension is placed under a coverslip on the hemocytometer. The hemocytometer is placed under a microscope and cells are counted in a designated area (e.g., small black box above). Calculations based on the dilution of cells are made to determine the starting concentration of cells. Some instruments are now available for automated counting.



Cell Culture Workflow

- Day 1

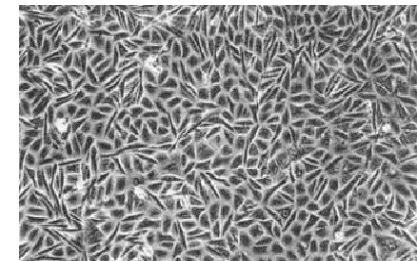
- Obtain cells in frozen state (only if using immortal cells; primary cells are received fresh)
- Thaw cells in cryovial in 37°C water bath; wash once in fresh media (spin to collect cells)
- Transfer to appropriate vessel and incubate overnight
(Note: Cells can be checked after approximately 1 hr for an estimate of viability)



Day 1

- Day 2–4

- Look at adherent cells in the tissue culture flask using a low magnification microscope. Cells should be well spaced and form a single layer (monolayer) on the flask surface. Feed by removing media and replacing it with fresh media 2–3 times/week



Day 6–8
Confluent Cells

- Day 6–8

- Once cells become confluent (growing very close together and crowded), they must be subcultured/split/passaged



Splitting Cells

- Adherent cells
 - Remove media from the tissue culture flask
 - Add a small volume (1–2 ml) of a mixture of trypsin in media or PBS to the flask, coating the cells. (EDTA is sometimes used in the place of trypsin; alternatively cells can be physically removed using a spatula)
 - Incubate at 37°C for 2–5 min
 - Tap the flask to dislodge the cells
 - Add media (3–5 ml) to inhibit the trypsin
 - Remove and aliquot the cells; dilute and count the cells
 - An aliquot of this suspension is used to start a new culture
- Suspension cells
 - Remove and count an aliquot of cells
 - Start a new culture with an appropriate volume of new cells



Resources

- ATCC (American Type Culture Collection) — www.atcc.org/
- ECACC (European Collection of Cell Cultures) — www.hpacultures.org.uk/collections/ecacc.jsp/
- DSMZ (German Collection of Microorganisms and Cell Cultures) — www.dsmz.de/
- Gene Transfer Protocol Library — www.bio-rad.com/genetransferprotocols/



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