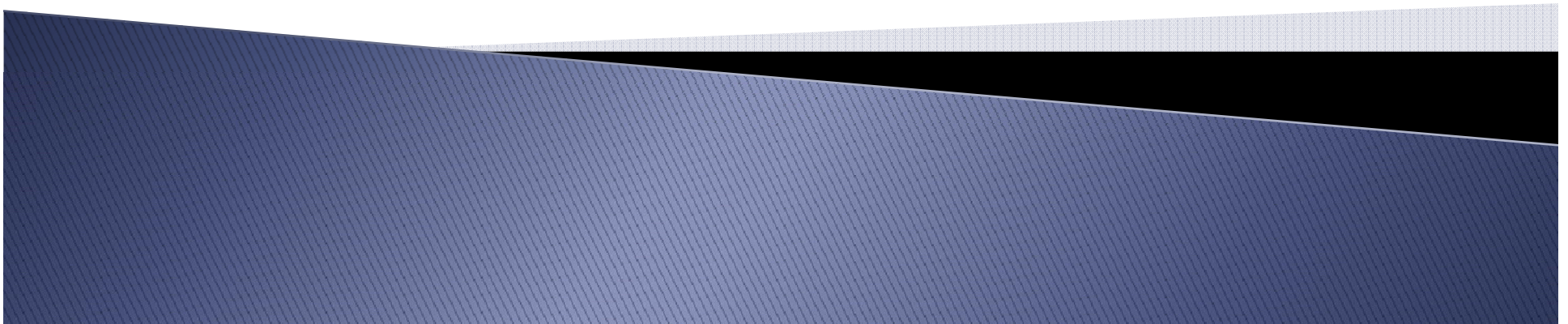


# CELL CULTURE TECHNIQUES

Bio4XX3

May 2009



# Types of cells in general use

- ▶ Classification by cell origin
  - Primary cells
  - Continuous cell lines
  - Hybridomas
  - Stem cells
- ▶ Classification by growth
  - Adherent , eg fibroblasts
  - Suspension, eg lymphoblasts

# Primary cells

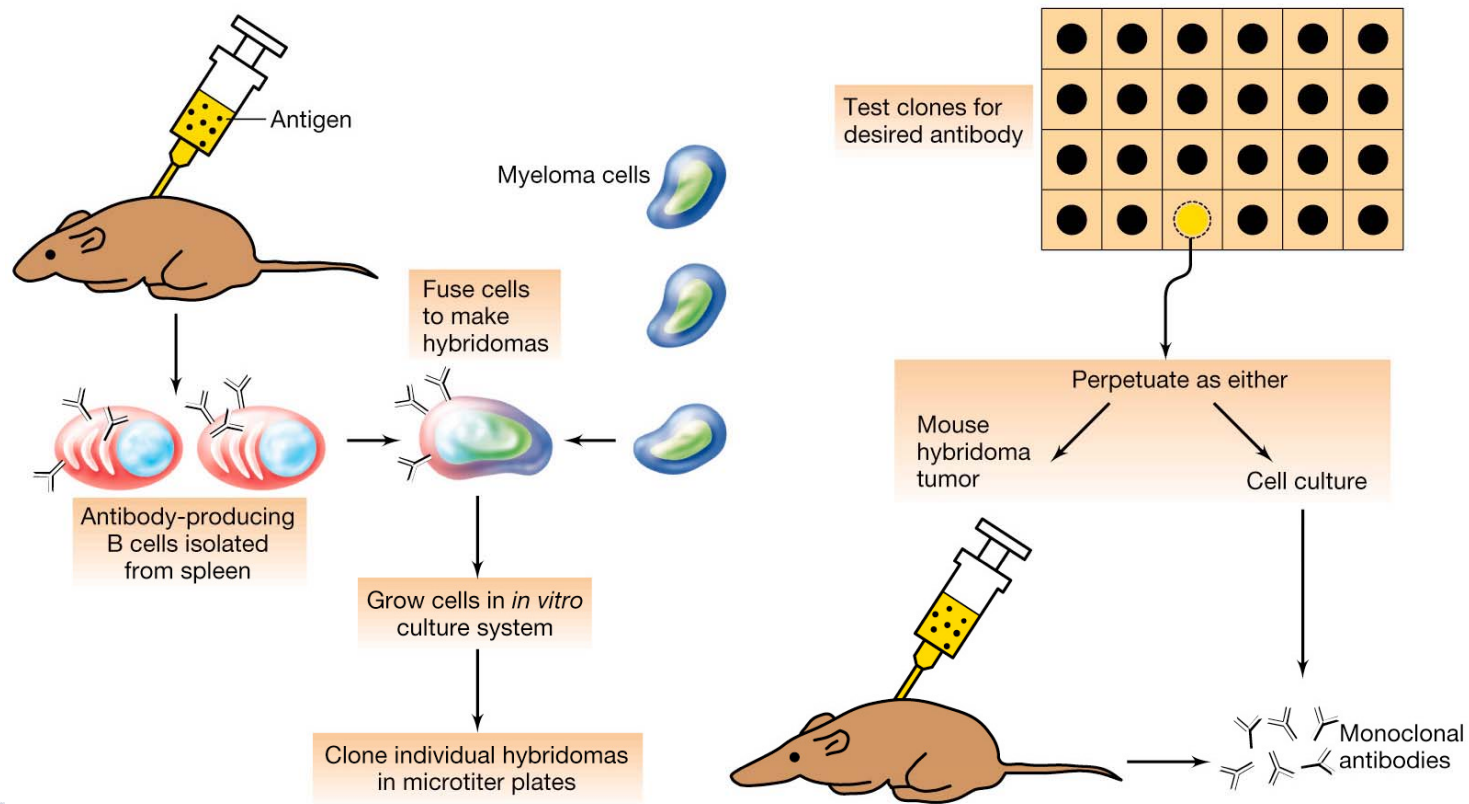
- ▶ Derived directly from animal tissues
- ▶ Disruption of tissue by mechanical and enzymatic means to release cells
- ▶ Limited life span (up to 50 doublings)
- ▶ Mixed cell population, mostly fibroblasts
- ▶ May become “immortalized” by spontaneous mutation or transformation
- ▶ Examples: MEF – mouse embryo fibroblasts  
CEF – chicken embryo fibroblasts

# Continuous cell lines

- ▶ Divide almost indefinitely
- ▶ Obtained from tumours, transformation by viral genes, spontaneous mutation from primary cell culture
- ▶ More homogeneous population
- ▶ Genetic and chromosomal abnormalities
- ▶ Some can be induced to differentiate in culture
- ▶ Examples: HeLa – human cervical carcinoma
  - 293 – adenovirus transformed
  - 3T3 – mouse immortal cell line
  - C2C12 – mouse myoblast cell line that can be differentiated into myotubes

# Hybridomas

- ▶ Specialized hybrid cell lines that produce antibodies to a defined antigen



# Stem cells

- ▶ Embryonic stem cells - pluripotent
  - ES cells obtained from the inner cell mass of cultured blastocysts
  - Require a feeder layer of cells (eg MEF) to grow
  - Mouse ES cells used to generate gene knockouts
  - Study differentiation
  - Human ES cells – therapeutic potential
- ▶ Adult stem cells – unipotent, multipotent
  - Differentiate along specific pathways
  - Skin, neural cells, hematopoietic cells



# Embryonic stem cells

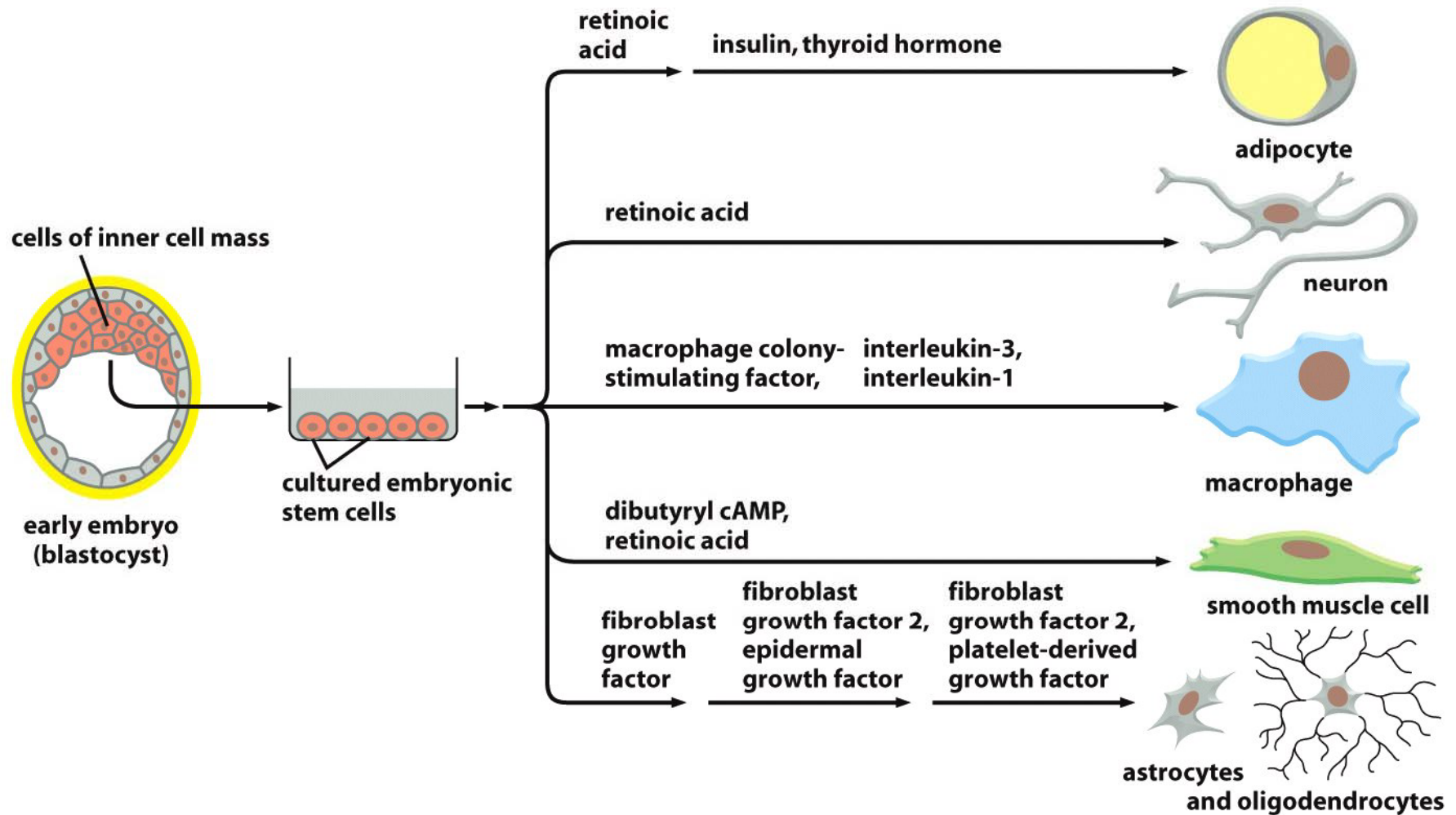


Figure 23-68 *Molecular Biology of the Cell* (© Garland Science 2008)

# Experimental uses of cell culture

- ▶ Transient transfection
  - DNA is introduced into cells, but is lost as the cells divide.
  - Examples: gene expression experiments, short term over-expression
- ▶ Stable transfection or transformation
  - DNA becomes integrated into the host cell genome (random, requires means for selection)
  - Genetically altered cell lines produced.
  - Long term studies
  - Examples: generation of viral packaging cell lines, GFP tagged proteins, gene knockouts

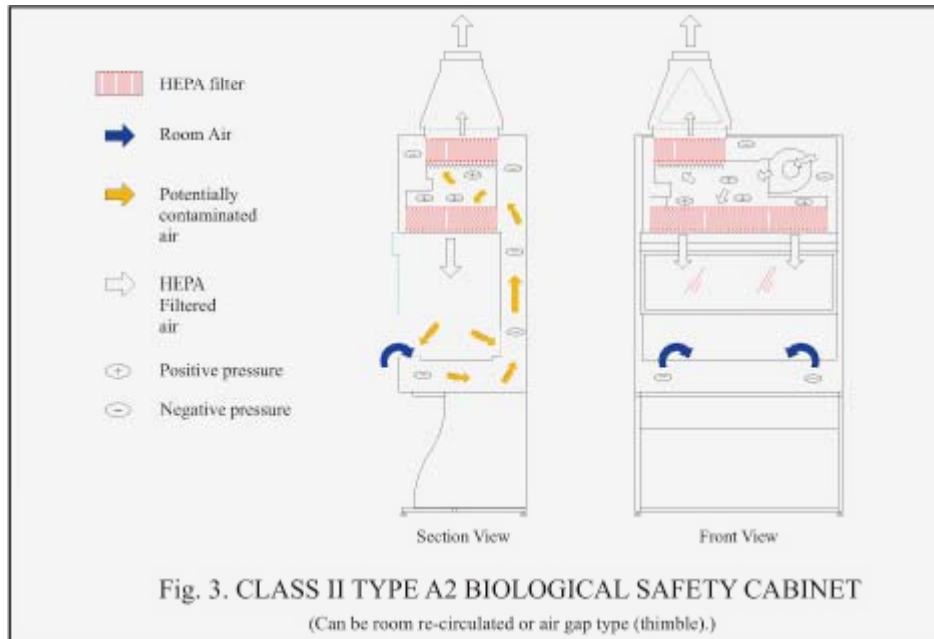


# Working with cell cultures

## Containment level depends on the risk factor determined by the Public Health Agency of Canada

- ▶ **Risk Group 1 (low individual and community risk)**
  - Any biological agent that is unlikely to cause disease in healthy workers or animals.
- ▶ **Risk Group 2 (moderate individual risk, low community risk)**
  - Any pathogen that can cause human disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available, and the risk of spread is limited.
- ▶ **Risk Group 3 (high individual risk, low community risk)**
  - Any pathogen that usually causes serious human disease or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that causes diseases treatable by antimicrobial or antiparasitic agents.
- ▶ **Risk Group 4 (high individual risk, high community risk)**
  - Any pathogen that usually produces very serious human disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa, directly or indirectly, or by casual contact.
- ▶ <http://www.phac-aspc.gc.ca/ols-bsl/>

# Work in the Biosafety cabinet



Airflow maintains sterile environment in the cabinet. Items introduced from outside are not sterile (hands, bottles, etc)

# Reagents required

- ▶ PBS – isotonic salt solution for washing cells.
- ▶ Culture medium eg DMEM (Dulbecco's modified Eagle medium)
  - Serum eg fetal calf serum, calf serum, horse serum (supplies growth factors)
  - L-glutamine (sometimes added separately)
  - Antibiotics and/or fungicides (help maintain sterile cultures)
- ▶ Trypsin or trypsin/EDTA solution used to dissociate adherent cells
- ▶ Sterile plasticware – petri dishes, pipettes, gloves

# Basic culture medium



MEM.htm

# Common cell lines

- ▶ HeLa – human, epithelial, tumour derived BSL2
- ▶ 3T3 – mouse, fibroblast, immortal BSL1
- ▶ Sf9 – insect, used for baculovirus production BSL1
- ▶ HEK293 – human, epithelial, Ad5 transformed BSL2
- ▶ COS – monkey, epithelial, SV40 transformed BSL2
- ▶ Jurkat – human, T cell leukemia BSL2
- ▶ Rat 1 – rat, fibroblast BSL1
- ▶ S2 – drosophila cell line BSL1
- ▶ CEF – chicken, fibroblast, primary BSL1

# Storage of cell lines

- ▶ Kept frozen in liquid N<sub>2</sub> (-170°C) in small aliquots (1ml)
- ▶ Freezing medium is growth medium + 10% glycerol or DMSO
- ▶ Freeze slowly – thaw quickly
- ▶ “Early passage” cells – cells frozen very soon after initial isolation
- ▶ Ideally only uncontaminated cells should be frozen!
- ▶ Stable for many, many years if storage conditions are monitored

# Problems - contamination

- ▶ Yeast – most common, cloudy medium, visible under the microscope, smells fermented
- ▶ Bacteria – not so common, cloudy medium, perform Gram's stain to confirm
- ▶ Mycoplasma – very common, intracellular parasites, no visible sign of contamination, cannot be readily cultured, special kits for detection

If you don't test your cell lines for mycoplasma then they are probably contaminated. Source is lab personnel and then very easily transmitted through contaminated media and pipettes

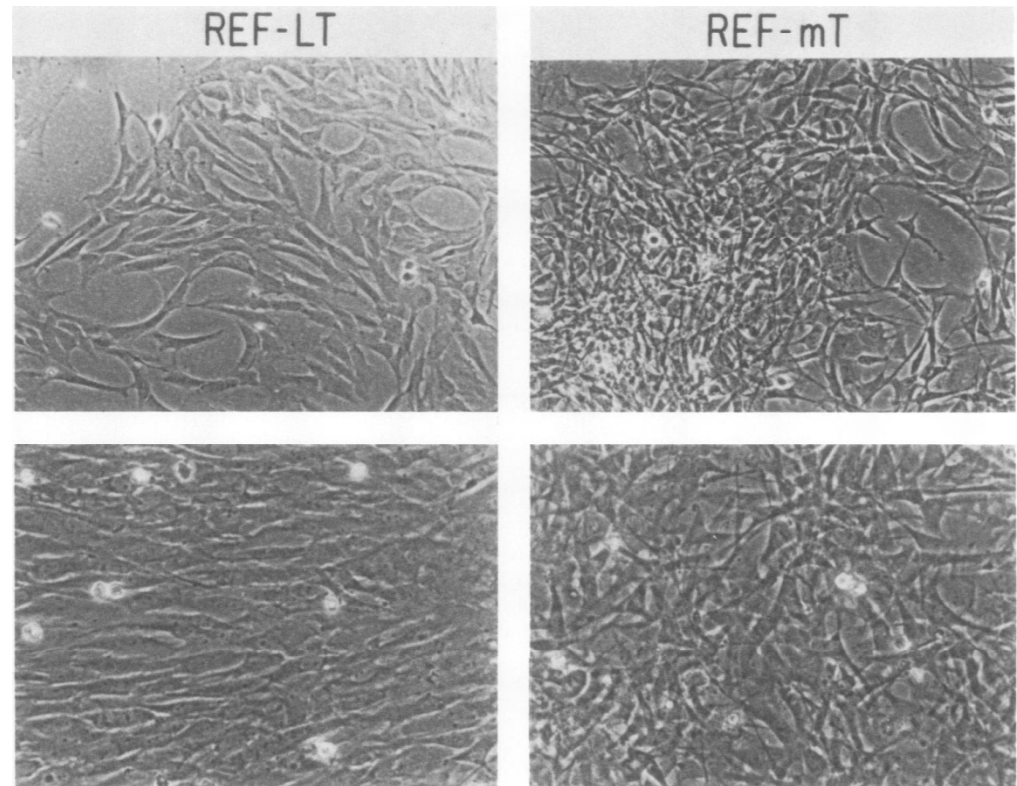


# Splitting/passing/passaging cells

- ▶ CEF cells – primary chick embryo fibroblast cells
- ▶ Rat 1 – immortal rat fibroblast cell line
- ▶ Confluent plate – no space between the cells – means cells must be diluted
- ▶ Media is removed and cells washed to remove traces of serum
- ▶ Treat with trypsin solution to detach cells from plastic
- ▶ Remove from the plate with complete medium and dispense small volume into fresh plates
- ▶ Dilution factors between 1:2 and 1:20 depending on cell type

# Rotation activity

- ▶ Subculture 1 confluent dish of cells into 2 fresh dishes at a 1:5 dilution
- ▶ Observe growth over the next few days
- ▶ If you are male you will be doing CEF cells
- ▶ If you are female – Rat 1 cells



Cowie et al. MCB1986