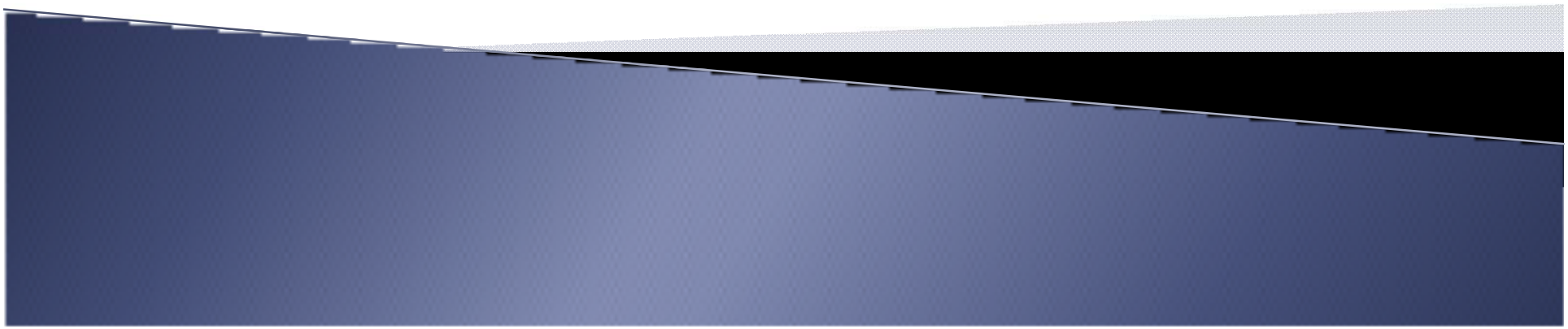
CELL CULTURE TECHNIQUES

MolBio4XX3 **May 2010**



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

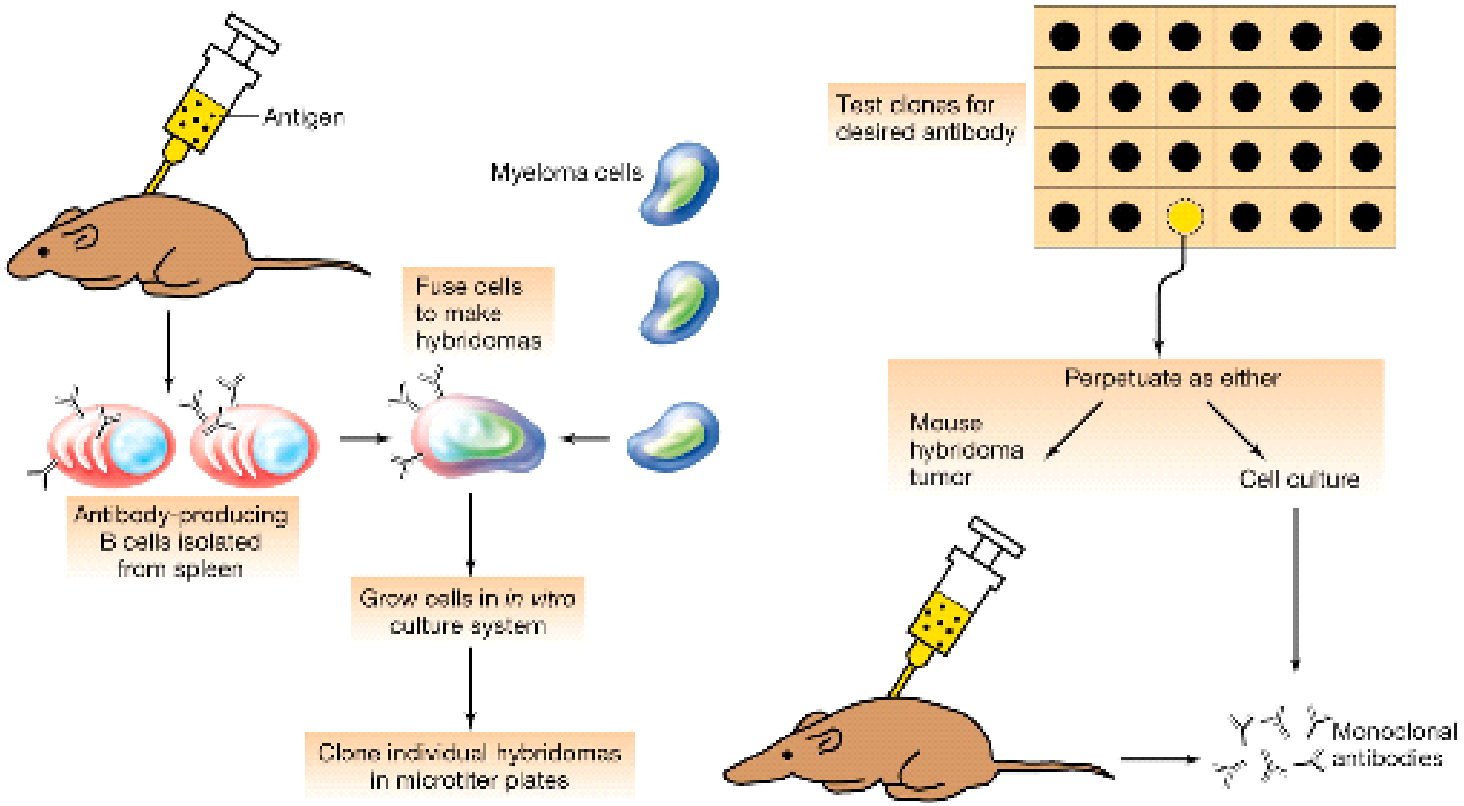
- Derived directly from animal tissues
- Tissue disrupted by mechanical and enzymatic means to release cells
- Limited life span (up to 50 doublings)
- Mixed cell population, mostly fibroblasts
- May become “immortalized” by spontaneous mutation or transformation
- Examples: MEF – mouse embryo fibroblasts
  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
  HEK293 – adenovirus transformed human
  3T3 – mouse immortal fibroblast 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, hematopoitic cells

Embryonic stem cells

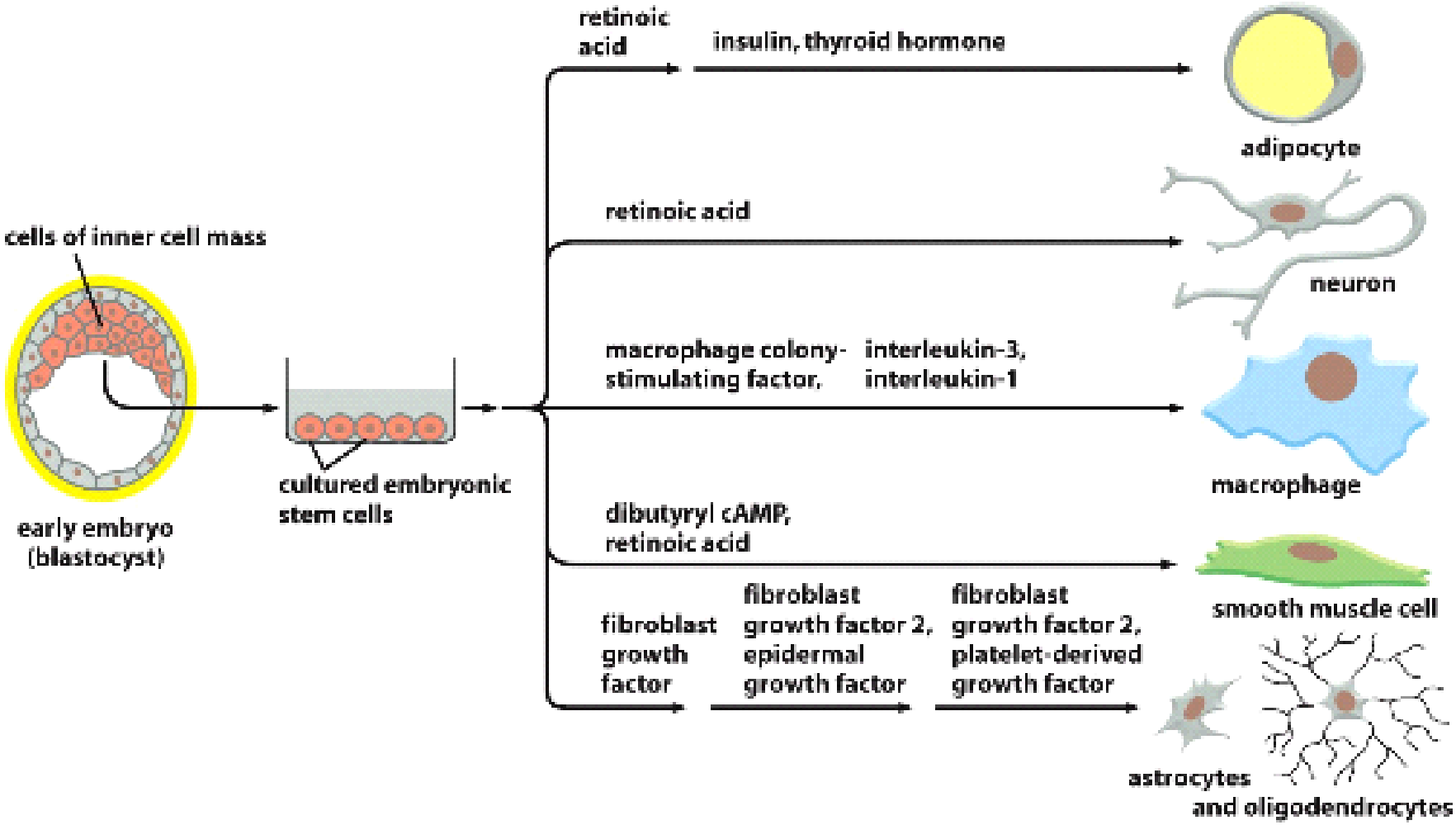


Figure 23-68 *Molecular Biology of the Cell* (© Garland Science 2008)

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 (requires site specific integration)

DNA transfection methods

- Calcium phosphate precipitation
  - Developed by Silvia Bacchetti and Frank Graham
  - Mixing DNA with calcium chloride and sodium phosphate to form a fine precipitate
  - DNA enters cell by engulfment of particles
  - Very cheap, OK efficiency, suitable for transient and stable transfection
- DEAE dextran solution
  - Diethylaminoethyl dextran +vely charged molecule binds to –vely charged DNA, attach to cell membrane and endocytosed
  - Cheap, OK efficiency, works best for transient transfection

More DNA transfection methods

- Electroporation
  - Electric current makes aqueous pores in cell membrane so DNA can enter
  - Needs special equipment: power supply and cuvettes
  - Efficient but results in a lot of cell death
- Lipofection
  - DNA becomes trapped in synthetic liposomes that fuse with the cell membrane
  - Very efficient, suitable for both types of transfection
  - Expensive, proprietory kits required

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

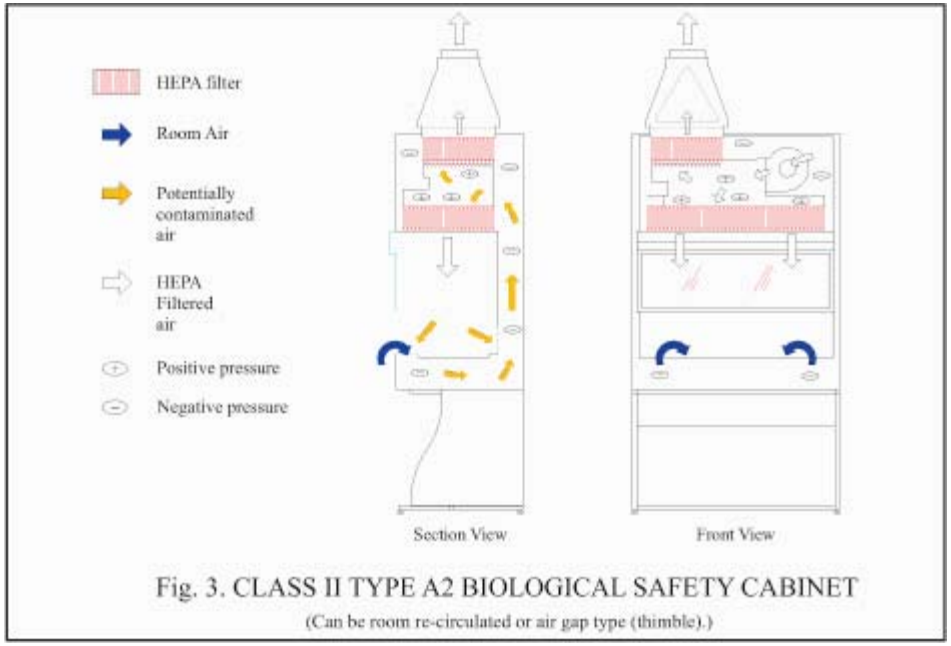


Fig. 3. CLASS II TYPE A2 BIOLOGICAL SAFETY CABINET

(Can be room re-circulated or air gap type (thimble).)

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

Reagents required

- PBS – phosphate buffered saline, 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 (to maintain "sterile" cultures)
- Trypsin or trypsin/EDTA solution used to dissociate adherent cells from the plastic and themselves
- Sterile plasticware – petri dishes, pipettes, gloves

Basic culture medium

Dulbecco's modified Eagle medium

inorganic salts	mg/L
CaCl2 (anhydrous)	200
Fe(NO3)·9H2O	0.1
KCl	400
MgSO4 (anhydrous)	97.67
NaCl	6400
NaH2PO4·H2O	125
NaHCO3	3700

amino acids	mg/L
L-Arginine·HCl	84
L-Cystine 2HCl	62.57
L-Glutamine	584
Glycine	30
L-Histidine HCl·H2O	42
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine HCl	146.2
L-Methionine	30
L-Phenylalanine	66
L-Serine	42
L-Threonine	95.2
L-Tryptophan	16
L-Tyrosine 2Na·2H2O	103.79
L-Valine	93.6

vitamins	mg/L
D-Ca Pantothenate	4
Choline Chloride	4
Folic Acid	4
Myo-Inositol	7
Niacinamide	4
Pyridoxine HCl	4
Riboflavin	0.4
Thiamine HCl	4

other	mg/ml
D-Glucose	4500
Sodium pyruvate	110
Phenol Red (Sodium)	15.9

http://cnhri.pcking.net/Source/DMEM.htm

NaHCO3 is buffering system

phenol red is pH indicator

orangey-red = OK

yellow = acidic (old culture, bacterial contamination)

purpley-pink = alkaline (too long out of incubator, yeast contamination)

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_2 (-170^{o}C) in small aliquots (1ml)
- Freezing medium is growth medium + 10% glycerol or DMSO
- Freeze slowly – thaw quickly, to avoid cell damage
- “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 – 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

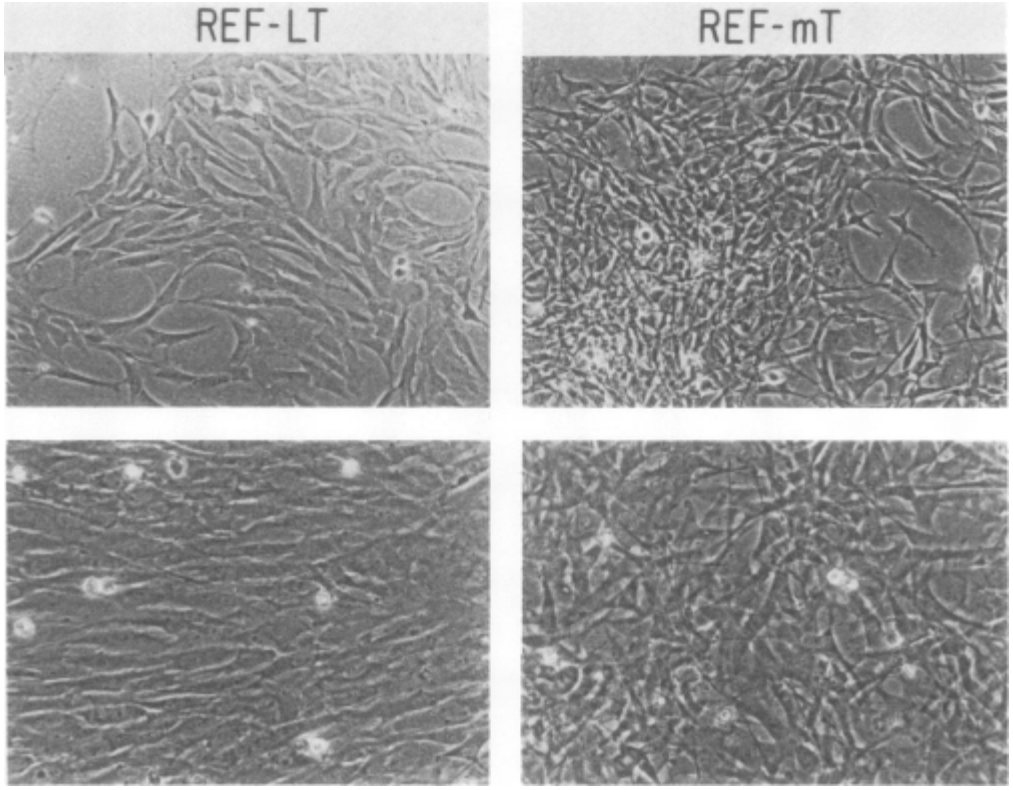
Splitting/passing/passaging cells

- Confluent plate – no space between the cells – means cells must be diluted
- Media is removed and cells washed to remove traces of serum (will inhibit trypsin)
- 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

- REF-LT: immortal, contact inhibited
- REF-mT: transformed by oncogene



Cowie et al. MCB1986