Animal Tissue Culture

SQG 3242

Primary culture, subculture, cell lines and cryopreservation

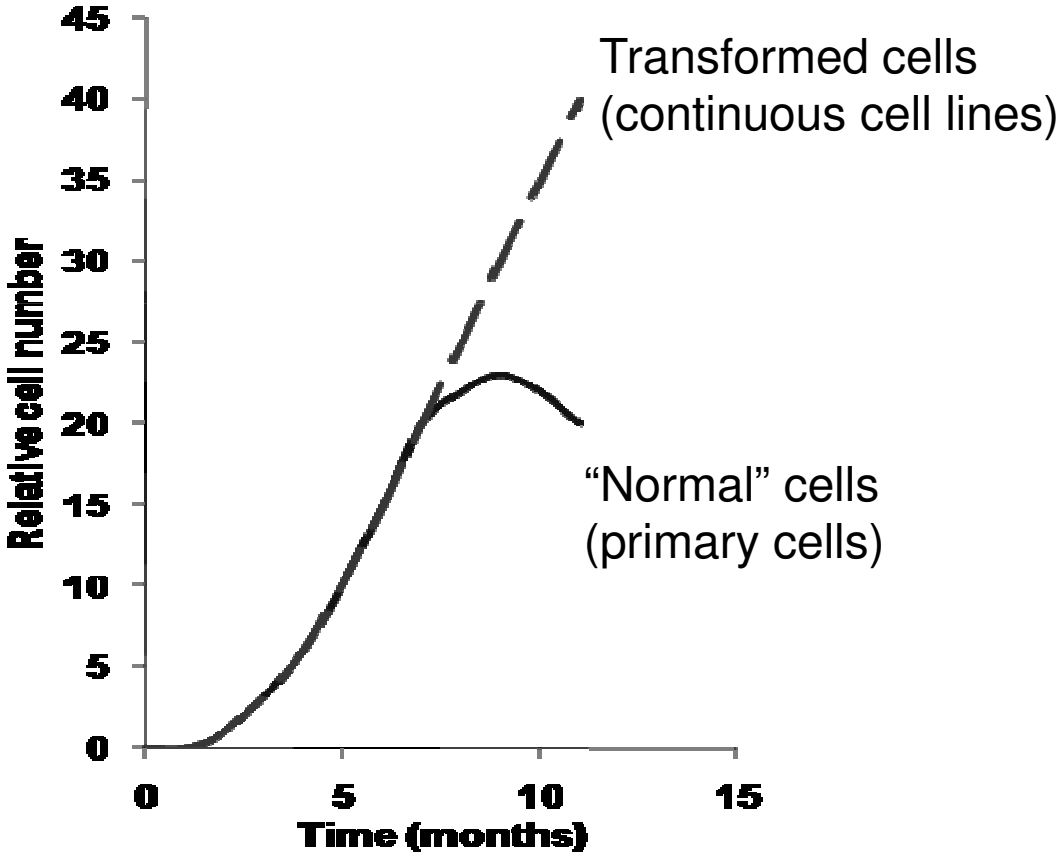
DR. SITI PAULIENA MOHD BOHARI

Classification of Cell Cultures

- **Primary Culture**
  - Cells taken directly from a tissue to a dish
  - Can be passages with a limited number of times. After the limit, the cell will die.

- **Culture of establish cell lines**
  - Established or immortal cell lines
  - Cells taken from a primary culture and passed or divided *in vitro.*
  - Can grow indefinitely in culture

Growth of normal and transformed cells in culture



Primary cells vs Est cell lines

Primary Cells

- Freshly isolated cells (from tissues)
- Hard to culture
- Heterogeneous (mixed) cell population (+ fibroblasts?)
- Need to try and purify/ remove fibroblasts?
- Finite number of passages (gradual loss of function?)
- More “physiological”?

Cell lines

- Originally primary, but then transformed so keep growing
- Easy to culture
- Homogeneous (cloned?) cell population
- Infinite number of passages (cancer-like)
- Less “physiological”?

Cell vs tissue culture

Cells

- Isolated cells
- Pure or mixed cell types
- Either in suspension or stuck to plastic
- Primary cells vs cell lines

Tissues

- Small chunks of tissue
- Mixed cell types
- “Floating” in media (not stuck)
- More “physiological”?

Passage and Freezing

- Culture on plates/flasks until confluent
- Harvest using trypsin/EDTA
- Collect cells at bottom of tube (centrifuge)
- Either/or
  - Resuspend in media and transfer to another plate/flask (passage)
  - Resuspend in freezing media

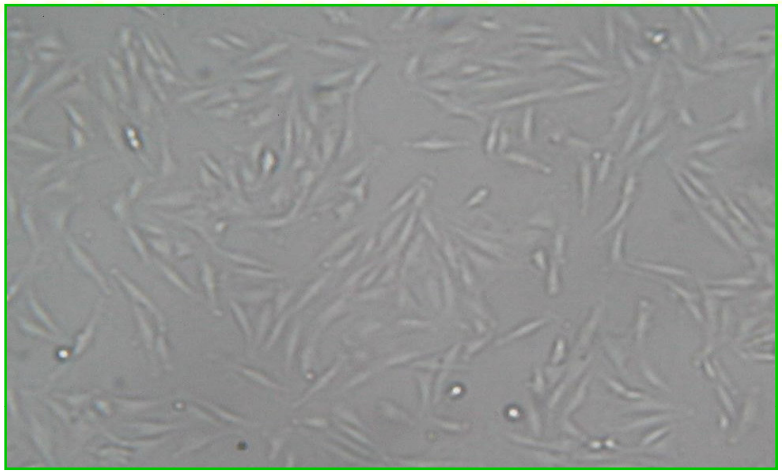
(+ dimethyl sulphoxide, DMSO) and freeze in liquid nitrogen

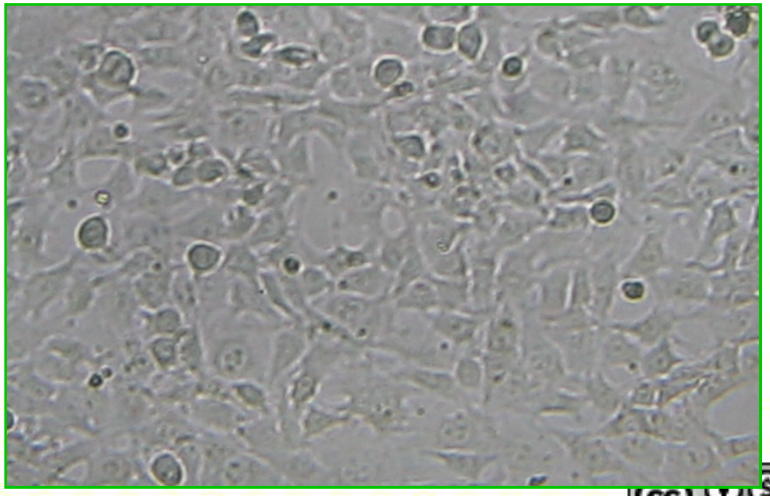
Understanding Cell Behavior

- **Confluency**

- How "covered" the growing surface appears

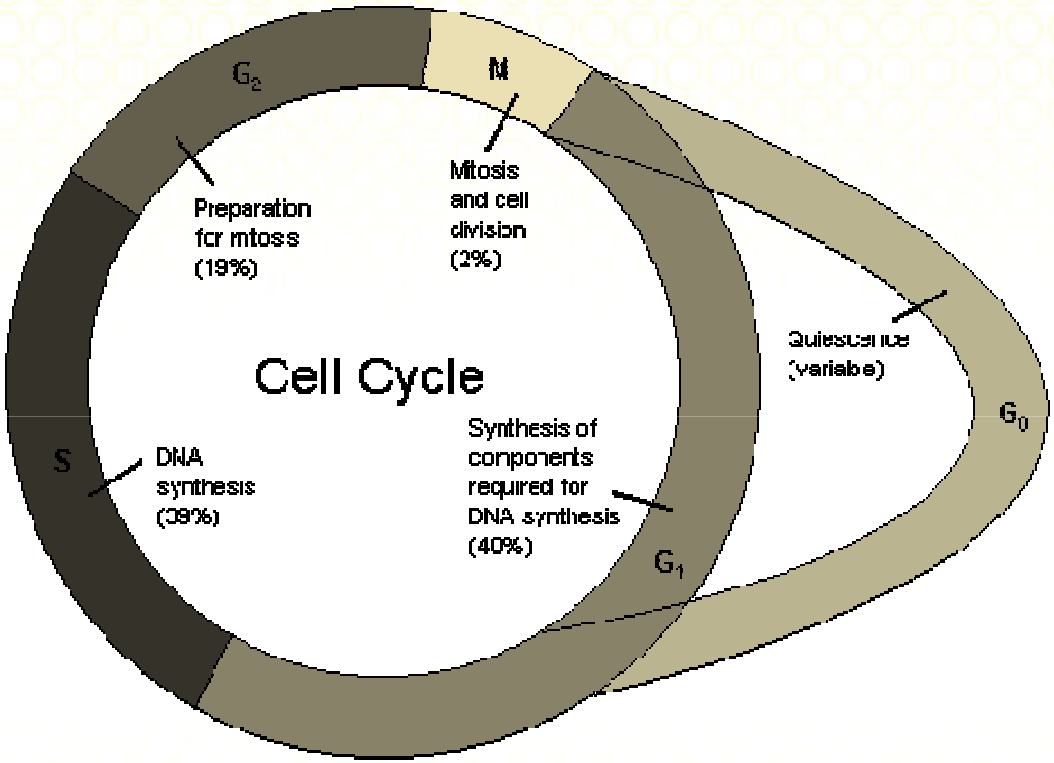
- This is usually a guess
- Optimal confluency for moving cells to a new dish is 70-80%
  - too low, cells will be in lag phase and won't proliferate
  - Too high and cells may undergo unfavorable changes and will be difficult to remove from plate.





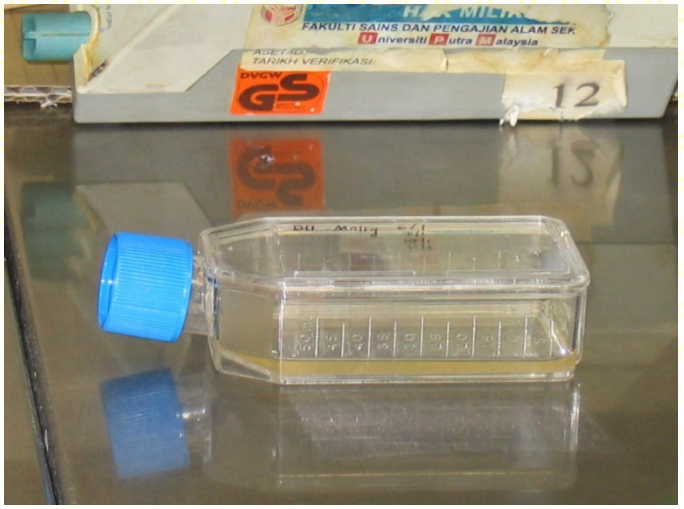
Contact Inhibition

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



Passaging cells

- Passaging or splitting cells involves transferring a small number of cells into a new vessel.
- Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density.
- Suspension cultures are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media.
- For adherent cultures, cells first need to be detached; this is commonly done with a mixture of trypsin-EDTA, however other enzyme mixes are now available for this purpose. A small number of detached cells can then be used to seed a new culture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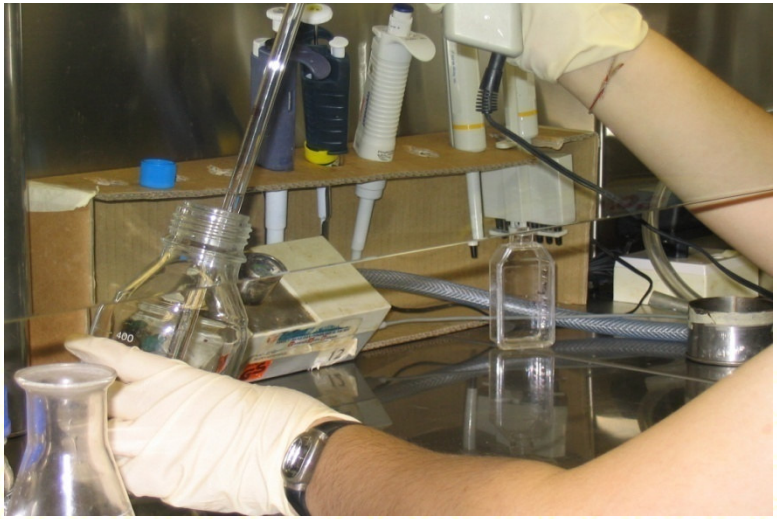

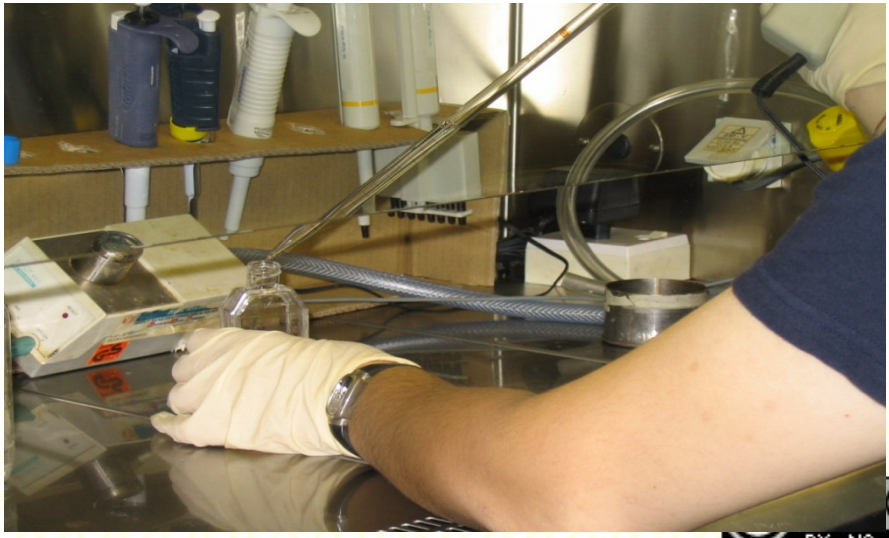

- **Anchorage Dependence**
- Cells that attach to surfaces *in vivo* require a surface to attach to *in vitro*.
  - Other cells or specially treated plastic or other biologically active coatings
- Blood cells are primary exception.
- Transformed cells may not require attachment.

- **Passage number**
- The number of times the cells have been removed (or “split”) from the plate and re-plated.
- Always write this on your plate or flask as P#

Improved titer of virus obtained by another technique:

Human diploid fibroblasts cellulose fiber micro carriers using suspension culture technique.

Growing importance of animal cell cultures for mass-production of certain proteins. One of it is monoclonal antibody obtained from hybridoma cells. Another proteins for therapeutic use, e.g growth factors, immunomodulators or hormones produced in recombinant cell lines.

These proteins have to undergo post-translational modification e.g. correct glycosylation that cannot be performed in recombinant microorganisms.

Maintaining cells in culture

- Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO2) in a cell incubator.
- Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed
- Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrient components.
- The growth factors used to supplement media are often derived from animal blood, such as calf serum. These blood-derived ingredients pose the potential for contamination of derived pharmaceutical products with viruses or prions.

Initiation of the culture

- A culture is derived either by the outgrowth of migrating cells from a fragment of tissue or by enzymatic or mechanical dispersal of the tissue
- Primary culture is the first in a series of selective process that may ultimately give rise to a relatively uniform cell line.
- In primary explantation, selection occurs by virtue of the cells capacity to migrate from the explants, whereas the dispersed cells only those cells that both survive the disaggregation technique and adhere to the substrate or survive in suspension will form basis primary culture.
- Selection of cells - Cells that capable of proliferation will increase, while some cells will survive but not increase and some are not survive.
- Cell confluent – the cells make close contact to each other
- Keeping cells density low-preserve the normal phenotype in culture.

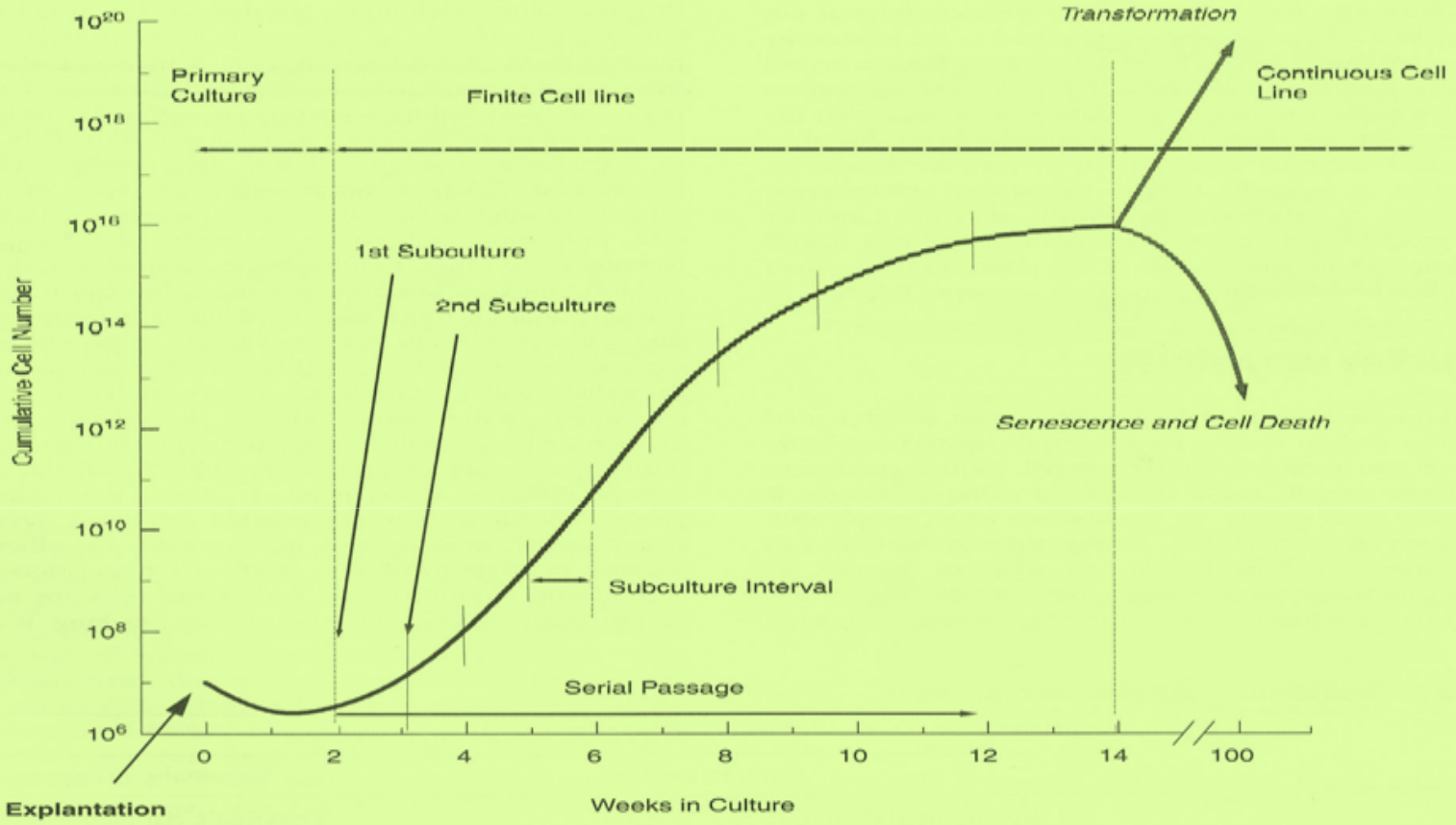

Fig. 3.8. Evolution of a Cell Line. The vertical (*Y*) axis represents total cell growth (assuming no reduction at passage) for a hypothetical cell culture. Total cell number (cell yield) is represented on this axis on a log scale, and the time in culture is shown on the *X*-axis on a linear scale. Although a continuous cell line is depicted as arising at 14 weeks, with different cells it could arise at any time. Likewise, senescence may occur at any time, but for human diploid fibroblasts it is most likely to occur between 30 and 60 cell doublings, or 10 to 20 weeks, depending on the doubling time. Terms and definitions used are as in the glossary. (After Hayflick and Moorhead [1961].)

Senescence

- Normal cells can divide a limited number of times, hence cell lines derived from normal tissue will die out after a fixed number of population doubling

- This is genetically determined event involving several different genes and is known as senescence

The development of continuous cell lines

- The ability of some cell lines to grow continuously probably reflect to its capacity or genetic variation, allowing subsequent selection.
- Genetic variation-involves the deletion and mutation of p53 gene which normally arrest the cell cycle progression
- A common feature of many human continuous cell lines is the development o a subtetraploid chromosome number.
- The alteration in a culture that gives rise to continuous cell line is commonly called in vitro transformation.
- Immortalization - the acquisition of an infinite lifespan
- Transformation - implies an additional alteration in growth characteristic

- Continuous cell line is usually uneuploid and often have chromosome number between diploid and tetraploid
- There is considerable variation in chromosome number and constitution among cell population(heteroploidy)
- Transformation has been applied to the process of formation continuous cell lines partly because the culture undergo morphological and kinetic alteration, but also because its is also accompanied by tumorigenicity.
- Many normal cells do not give rise to continuous cell lines.

- The source of the culture will also determine which cellular component may be present.

- Cell lines derived from embryo
  - contain more stem cells and precursor cells
  - Greater self renewal than culture from adult
- Culture from tissue
  - Undergoing continuous renewal *in vivo*
  - May still contain stem cells under appropriate condition
  - Prolonged life span
  - Culture from tissue that renew solely under stress, may contain only committed precursors with limited life span

- The identity of cells is defined by its lineage in vivo and by its position in that lineage

Pharmaceutical Application of Animal Cell Cultures

The important commercial utilization of animal cell cultures is for multiplication of viruses for manufacturing of vaccines.

Viruses require living substrate for multiplication. Previously, duck embryos or organ cultures from animal nerves or kidney tissues were used for virus vaccines. Such explants have different types of cells with different demands on the medium. Another disadvantage comprehensive sterility test must be done. Also kidneys from wild apes have simian viruses (SV-5, SV-40) or their constituents. Characterized cell cultures under sterile conditions provide decisive advantage.

Production of polio vaccines by replication of virus on simian kidney cells have been carried out by Megneir et al (1979). After attaching primary cells to support, the primary cells are grown for 7-8 days with no change in medium. In this phase sterility test and chromosome analysis can be carried out. Three days after infection by polio virus, culture is ready for preparation of vaccine. Vaccine prepared by concentration, chromatography method (Sepharose 6B & DEAE Sephadex) and inactivation (formaldehyde) of virus.

References

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