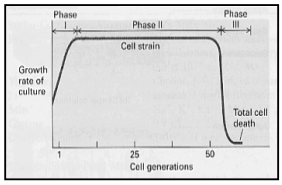
Primary culture

Classification of tissue cultures based on the origin of the cells

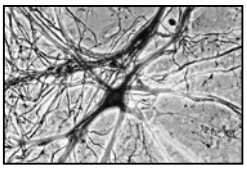
- Primary culture (directly from animal or plant tissue)
- Extended culture (multipassage culture) – cell strain
- Established (transformed) cell lines

Primary tissue culture

- A culture derived directly from a tissue
  - A stage from cell isolation to first subculturing
  - Carrot callus growth was primary plant culture
- Best resembling natural tissue
- Limited growth potential
- Limited life span
- May give rise to a cell strain or be immortalized
- Strain – a lineage of cells originating from one primary culture



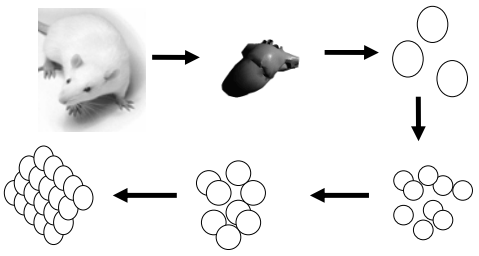
Survival times of primary neuronal cells in culture



Tissue	No. of Experiments	Maximum Survival Time in vitro	Size Range of Somata
spinal cord	792	312 d.i.v.	10 - 45 µm
cortex (frontal)	58	81 d.i.v.	----
cortex (auditory)	56	148 d.i.v	5 - 17 µm
olfactory bulb	67	172 d.i.v.	----
cerebellum	6	---	---
hippocampus	4	---	---

Steps in primary tissue culture

- Isolation of tissue
- Disaggregation of cells – initiation of culture
- Incubation and growth



Isolation of tissue

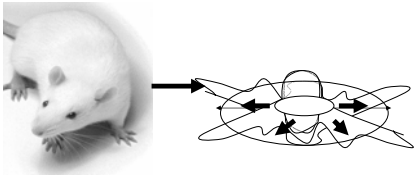
- Make sure your work is within rules
- Work safely, especially with human tissue
- If you isolate your cells far from culture place (as it is in our case) keep it on ice (4ºC) for up to 72 hours

Disaggregation of cells

- Cells can be allowed to migrate out from an explant
- Mechanical dissociation (mincing)
- Enzymatic dissociation

Exception – hematopoietic cells do not need to be disaggregated, they already are

Explant culture



Explant culture

- Involves placing a piece of tissue into the tissue culture dish and allowing cells to migrate out from the tissue
- First type of cell culture developed
- Performed in the case of cells which are protease sensitive
  - Smooth muscle cells, bone cells
- Or in case of small amount of tissue (such as needle biopsies)
- Not very effective for cells with poor adhesion (migration)
- Fibrinogen and thrombin used to stimulate adhesion
- Disadvantages – selection by speed of migration, type of attachment, localization within tissue etc.

Enzymatic disaggregation

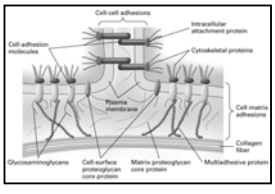
- Avoids selection of cells by migration and usually yields more representative sample
  - But still selects by resistance to enzymatic treatment
- Faster than explant

Enzymatic disaggregation

- Cell to cell adhesion is mediated by a variety of cell adhesion molecules
- The connections between cells and extracellular matrix have to be broken
- To break calcium dependent adhesion (cadherins and selectins) we use EDTA or EGTA (both calcium chelators)
- Extracellular matrix proteins such as fibronectin and laminin are protease sensitive
- Proteoglycans can be partially degraded by hyaluronidase or heparinase

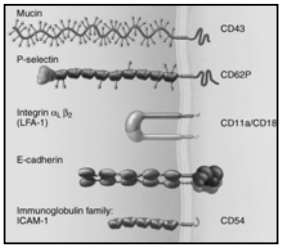
Physical connections between cells

- Cells in multicellular organisms are in contact with each other or extracellular matrix
- Cell connections involve multiple ligands and cell adhesion receptors
- The interaction between cell adhesion receptors and their ligands are relatively weak
  - A lot of weak interactions make a strong bond



Principal classes of cell-adhesion receptors

- Cadherins
- Ig-family of cell adhesion molecules
- Integrins
- Selectins
- Others such as
  - Mucins
  - Connexins



Extracellular matrix

- Network of proteins and carbohydrates that binds cells together
  - Supports and surrounds cells
  - Regulates cells activities
- Only 5 classes of macromolecules
  - Collagens
  - Elastic fibers
  - Proteoglycans
  - Hyaluronan
  - Adhesive glycoproteins
- They can be mixed up in different proportions for different functions

Enzymes used in enzymatic disaggregation

- Enzymes
  - Trypsin
  - Collagenase II (from Pseudomonas perfringens)
  - Elastase
  - Hyaluronidase
  - DNase
  - Pronase (bacterial protease)
- Usually a combination of enzymes
- Crude preparations are usually more efficient
  - The purer the less toxic
  - The cruder the more effective due to contamination with other proteases

More rules

- Start with trypsin/EDTA and than proceed to more complex enzymes
- Warm or cold trypsin
- Cold seems to give a higher yields but warm is faster (shorter exposure)
- If using warm trypsin collect cells every half hour to avoid cell death from exposure (remember to inactivate and remove trypsin before plating cells)
- Warm trypsin works better with big amounts of young tissue (mouse or chick embryos) and not too well with adult tissue (more connective tissue)

Cold trypsin

- Cold trypsin – avoids damage by exposure to warm trypsin
- Allows for enzyme penetration with minimum of enzymatic activity
- Followed by faster 37ºC digestion time
- Gives higher yield and higher survival rate
- Preserves more different cell types
- Convenient

Other enzymatic procedures

- Some tissues such as fibrous connective tissue are resistant to trypsin
- Collagenase – particularly connective tissue and muscle
- Hyaluronidase – to dissolve proteoglycans
- Pronase and dispase – bacterial proteases
- DNase – to dissolve DNA aggregates from damaged cells

Mechanical disaggregation

- Produces cell suspension quicker than other methods
- But causes more mechanical damage
- Several methods
  - Mincing
  - Collecting cells when tissue is sliced
  - Pressing the tissue through a series of sieves
  - Repeated pipetting

Incubation and growth

- Appropriate medium supplemented with growth factors, cytokines and all the goodies
- Some cells require special adhesion surfaces (cover tissue culture dish with extracellular matrix proteins or synthetic attachment molecules)
- Transfer cells to final growing conditions as soon as possible
- Challenges
  - Removal of dead cells
  - Enrichment of viable cells
  - Separation of cell types

Separation of nonviable cells

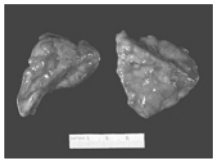
- For adherent cultures first change of media
- Gradual dilution of suspension cells when proliferation starts

Separation of cell types

- Selective media
- Difference in the speed of attachment
- Use of enzymes
  - Collagenase does not easily disperse epithelial cells but works well on stroma
- Neurons need NGF while glial cells don't

Adrenal Glands

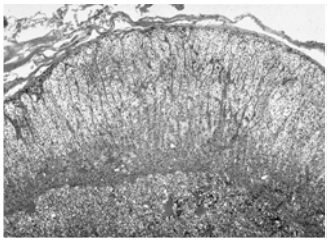
- Also called the suprarenal glands
- Located above the kidneys
- Highest blood flow rate in the body
- Two Regions
  - Medulla (inner)
  - Cortex (outer)



- Medulla & cortex are functionally different glands
  - Embryological origins:
    - Medulla derives from ectoderm (neural crest)
    - Cortex derives from mesoderm
  - Is actually two separate organs in amphibians

Histology of adrenal gland

- Produce glucocorticoids, mineralocorticoids, steroids, (cortex) and catecholamines (medulla)



Function of adrenal medulla

- Pheochromocytes also called chromaffin cells are modified, axonless neurons with a purely secretory function
  - Chromaffin cells because they stain dark with chromium
- Chromaffin cells produce primarily epinephrine (adrenaline), can also produce norepinephrine, but not both
  - Essential for fight or flight response
- Other hormones produced by the medulla are: dopamine, met-enkephalin (opioids), ADH, NPY, and adrenomedullin

Basic rules

- Remove fat and dead tissue
- Use sharp instruments for dissection to avoid cell damage
- Do not leave enzymes in the cell suspension
- Use high concentration of cells when seeding (much higher than for cell lines)
- Use appropriate, rich media
- Remember that primary cells might require very specific growth factors or attachment factors
- Embryonic tissue gives better results

Cold trypsin – the procedure

- Sterilize
- Remove fat and dead tissue
- Slice adrenal gland in half
- Cut out a piece of the medulla
- Wash it twice
- Add cold trypsin
- Cold incubation
- Remove trypsin
- Warm incubation
- Plating