KING'S College LONDON

INSTITUTE OF PSYCHIATRY,
PSYCHOLOGY & NEUROSCIENCE

Module:
Techniques in neuroscience

Week 4:
Tissue culture: Growing and studying neural cells in a dish

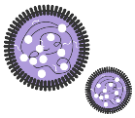
Dr Graham Cocks

Topic 1:
An introduction to tissue culture
Part 2 of 2

Part 2

Antibiotics

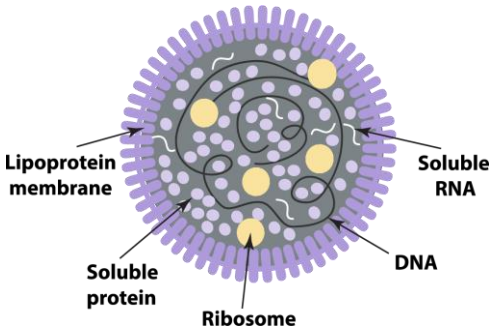
Widespread use of the antibiotics, penicillin and streptomycin in the 1940s onwards reduced the problem of microbial contamination of cultures.



These are ineffective against certain common strains of bacteria such as mycoplasma.

Mycoplasma

Mycoplasma are very small
(<1 micron in length)

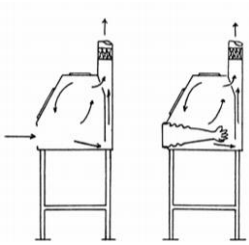


- can be a significant problem in long-term culture
- some antibiotics are effective against mycoplasmas
- best practice is to prevent contamination by employing an aseptic technique

Biological safety cabinets

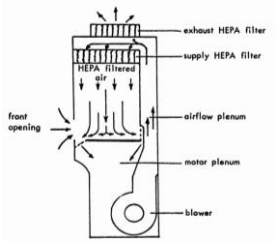
Biological safety cabinets are one of the most important developments in improving aseptic techniques.

Class I cabinets



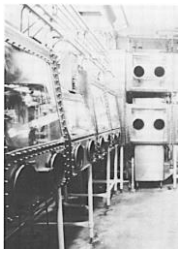
Characteristics:
- protects both the user and the environment from the sample
- does not protect the sample from airborne particles from the environment

Class II cabinets



Characteristics:
- protects sample from outside contamination
- relies on a continuous uniform flow of clean filtered air travelling down over the sample

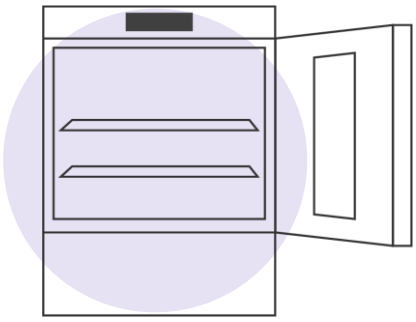
Class III cabinets



Characteristics:
- completely encloses the sample, which can only be accessed through the gloves integrated into the cabinet

Kruse et al. (1991)

Tissue culture incubators



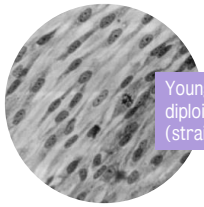
Tissue culture incubators maintain a **number of critical parameters** to **allow optimal growth and survival**:

- constant levels of temperature, humidity, CO_2 and oxygen
- most media currently in use for tissue culture use buffers that require an atmosphere of 5 per cent CO_2 to maintain a physiological pH
- some cell types grow better under low oxygen conditions – specific incubators can reduce oxygen levels with displacement by nitrogen

Primary cells, cell lines and cell strains

Leonard Hayflick & Paul Moorhead
(1961)

Derived the first strains of human fibroblasts (WI-38)



Young human diploid cells (strain WI-38)

Made the distinction between primary cells, cell lines and cell strains:

- primary cells are derived from normal tissue and grown without passaging
- cell strains are derived from primary cells which have a limited capacity for growth and division, but retain a normal karyotype
- cell lines have the capacity to grow indefinitely and invariably have abnormal karyotypes

Karyotype:
the size, shape, and number of chromosomes in a cell

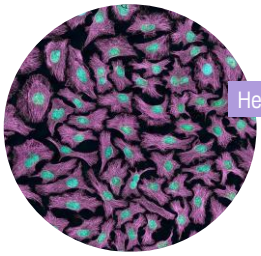
Hayflick & Moorhead (1961)

Immortal human cell lines

George Otto Gey
(1951)

Gey cultured cells from an individual called Henrietta Lacks who had cervical cancer.

Finding:
Cells derived from the cervical tumour could grow and divide indefinitely.

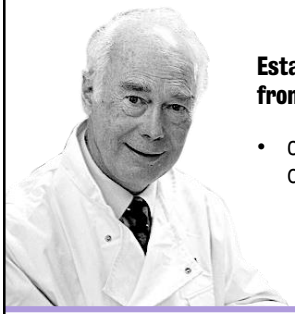


HeLa cells

- aided the development of the first Polio vaccine
- still being used for research today

Scherer et al. (1953)

Embryonic stem cells



Martin Evans
(1982)

Established cultures of cells derived from mouse blastocysts:

- can in principle generate any cell type of the body in a cell culture dish

First generated human embryonic stem cells from human blastocysts:

- allowed for the generation of inaccessible cell types, such as neurons, in large numbers for the first time
- ethical issues need to be considered

Jamie Thomson
(1998)

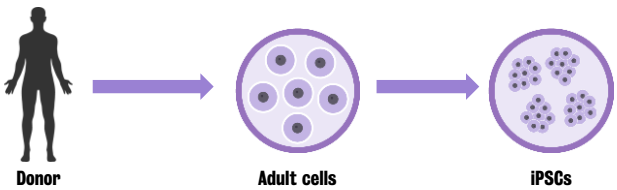
Evans & Kaufman (1981); Thomson et al. (1998)

Induced pluripotent stem cells

Shinya Yamanaka
(2006, 2007)

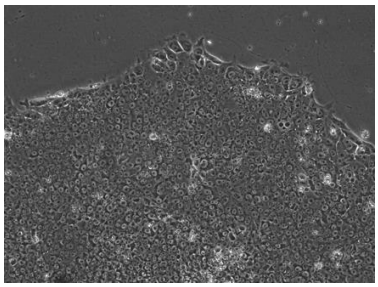
Directly generated embryonic stem cells by directly manipulating fibroblasts in a process called reprogramming:

- produced the so-called induced pluripotent stem cells (iPSCs)
- used to study genetic diseases and inaccessible cell types
- no ethical issues as those surrounding embryonic stem cells

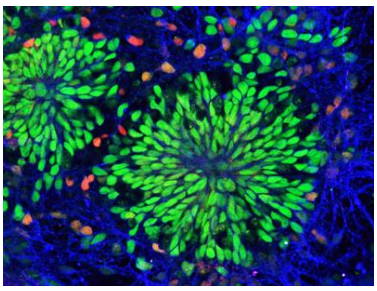


Takahashi et al. (2007); Takahashi & Yamanaka (2006)

Visual examples of iPS cells, neurons and neural 'rosettes'

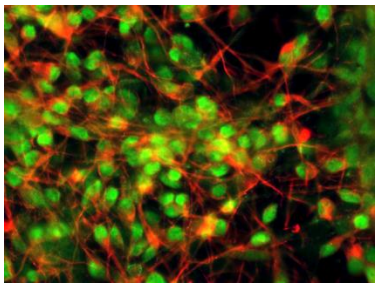


iPS cells growing in culture



Neural 'rosettes' surrounded by neurons

The neural rosettes are composed of radial glial cells (green) which in turn generate intermediate progenitors (red) which then in turn generate neurons (blue). These rosettes are considered to be a 2-D cell culture model of cortical neural tube development.



neurons fixed and stained with fluorescent markers

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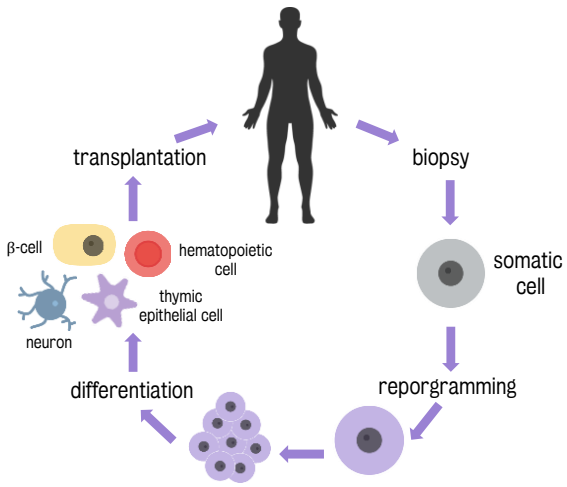
Regenerative medicine



Following on from the development of human iPSCs is the exciting prospect of using these cells for personalised **regenerative medicine**.

Major advantage:
Transplanted cells will be genetically identical to that individual, eliminating the risk of rejection or the use of immunosuppressive drugs.

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End of topic