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 1 of 2

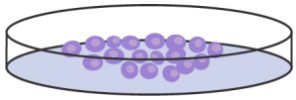
Topic list

This week, we will be looking at the following topics:

- ***Topic 1: An introduction to tissue culture***
- Topic 2: Video of procedures
- Topic 3: Focused journal club

Click **Next** to continue

Fundamentals of tissue culture



Historical perspective

Part 1

What is tissue culture?

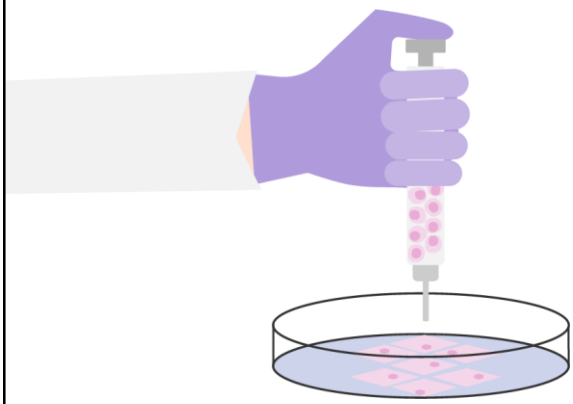
Tissue culture:
cultivation of eukaryotic tissues outside of the organism, in a growth media with the necessary nutrients, inorganic salts and pH required to function in a physiologically normal manner

'Cell culture':
culturing of dissociated cells rather than pieces of tissue

Why is tissue culture useful?

Useful as a model system for studying the basic processes of cell biology

and also has many clinical applications.



Examples of clinical applications:

- diagnosis of chromosomal disorders from the culture of blood or amniotic fluid samples
- generation of monoclonal antibodies for the production of vaccines as a result of the development of hybridoma cell lines by

Kohler & Milstein *(1975)*

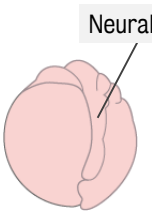
- in vitro fertilisation, through techniques developed for the culture of the early embryo, and first achieved by

Patrick Steptoe & Robert Edwards *(1977)*

Köhler & Milstein (1975); Steptoe & Edwards (1978)

Historical advance in tissue culture: *ex vivo* survival to early culturing

Maintained the neural folds from early chick embryos in a saline solution



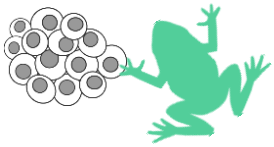
Wilhelm Roux ***(1885)***

Removed small section of frog embryos and embedded them in blood clots on the underside of coverslips to allow microscopic evaluation

Ross Granville Harrison ***(1907)***

Generated the first 'cell line' from embryonic chicken heart

Alexis Carrel & Montrose Thomas Burrows ***(1911)***



Carrel & Burrows (1911); Harrison et al. (1907); Jedrzejczak-Silicka (2017)

Development of growth media

Media based upon blood products gave rise to problems with reproducibility of results due its poorly defined nature.

Made first defined liquid media to try and overcome this problem:

- grew embryonic chick tissue in a relatively simple defined liquid media
- nowadays, cell types are still typically grown with media containing serum

The cultivation of tissues from chick embryos in solutions of NaCl, CaCl2, KC1 and NaHCO3

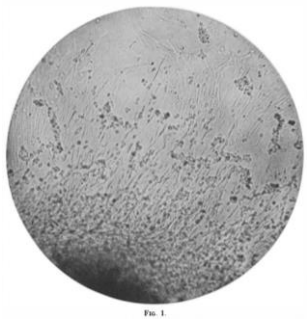
Margaret Reed Lewis & Warren H. Lewis **(1911)**

Lewis & Lewis (1911)

Enzymatic dissociation of tissue into cells and their 'passaging'

Francis Peyton Rous & F.S. Jones ***(1916)***

First demonstrated the use of proteolytic enzyme trypsin to dissociate tissues into individual cells for culture



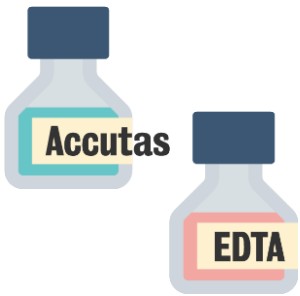
Most cell types, with the exception of blood cells, grow attached to an extracellular matrix (ECM):

- the extracellular matrix is composed of a complex mixture of polysaccharides and proteins such as collagens and laminin
- tissue culture vessels coated with purified of unpurified components of the ECM help to support attachment and normal functioning of many types of adherent cells
- cell adhesion molecules on the surface of many cells bind strongly to components of the ECM
- the use of trypsin also allowed for the re-plating of cells grown attached to a substrate

Rous & Jones (1916)

Passaging cells with alternatives to trypsin

Trypsin is still in use today to enzymatically dissociate tissues into single cells.



Gentler enzymes such as Accutase and non-enzymatic methods such as EDTA solutions are increasingly used.

Non-enzymatic methods chelate ions such as calcium that are essential for the function of cell adhesion molecules.

These methods tend to result in reduced cell death.

Freezing cells

Dissociated cells can be frozen indefinitely in liquid nitrogen.

Steps to freeze and thaw cells include:

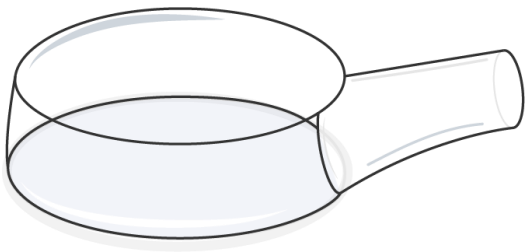
- cells are detached using the same methods used for passaging cells and re-suspended in a solution with a cryo-protectant such as DMSO (dimethyl sulfoxide)
- cells are frozen down initially in a -80 freezer at a rate of 1°C/minute using a vessel filled with isopropanol
- transfer cells to a liquid nitrogen vessel to be stored indefinitely
- cells are revived by rapidly thawing in a 37°C water-bath.

Culture vessels

Alexis Carrel & Lillian E. Baker *(1923)*

Developed a new vessel for tissue culture

The Carrel flask



Features:

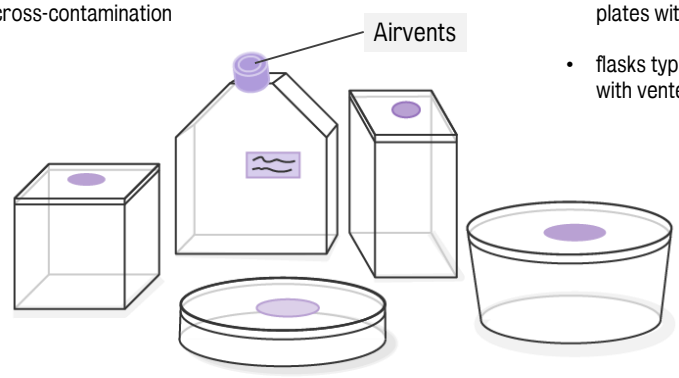
- angled neck to prevent airborne particles from settling into the flask when open
- allows for sterilisation with a flame further reducing the risk of airborne contaminants infecting the culture

Jedrzejczak-Silicka (2017)

Modern tissue culture vessels

Most modern tissue culture vessels are made of plastic, are sterile and are intended for single use:

- reduces the risk of microbial contamination and cross-contamination



Most tissue culture is performed in plates or flasks and these come in varying sizes:

- plates range from single dishes up to 15cm in diameter to plates with 384 wells
- flasks typically range in size from 25cmsq to 175cmsq, often with vented lids

End of part 1