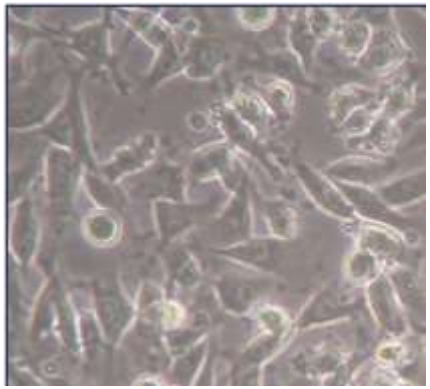
University of Leicester



Tissue Culture

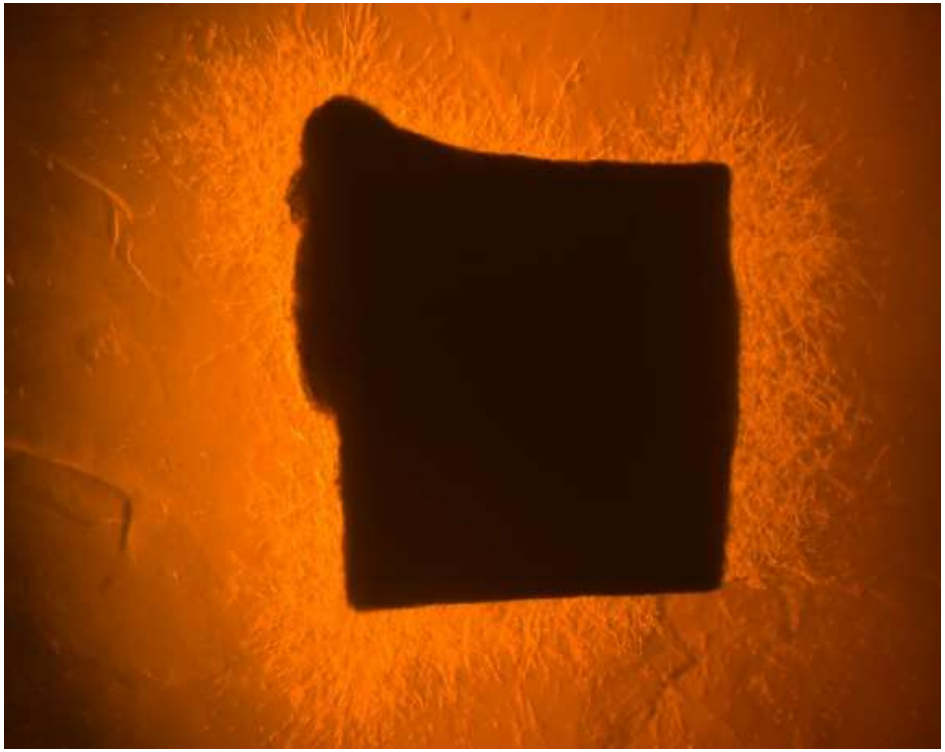
Dr Gina Barnett

Café Cardiologique
29th January 2014

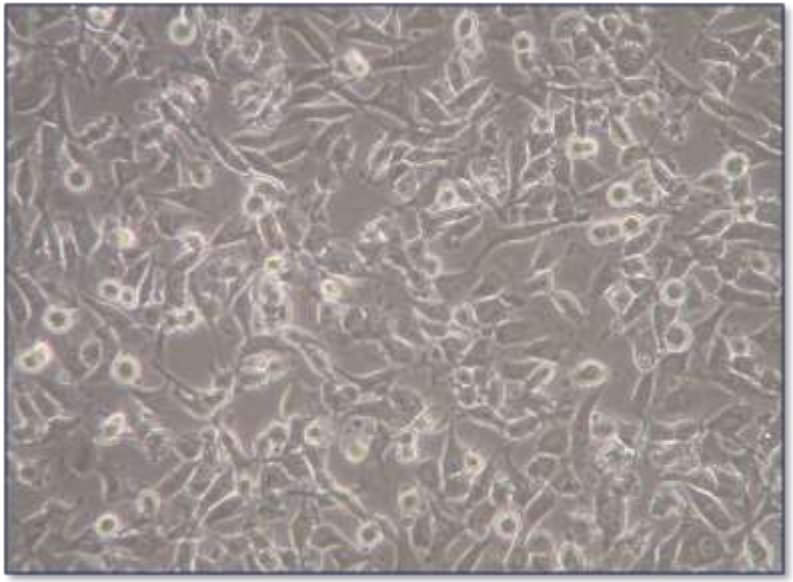
Tissue Culture

In vitro cultivation of:

- Organs (organ culture)
- Tissues (explant culture)
- Cells (cell culture)



Rat aortic explant culture



HUVEC Cells

Brief History of Tissue Culture

1885	Wilhelm Roux		Embryonic chick cells maintained alive in a saline solution
1907	Ross Harrison		Frog Embryo nerve fibre out**growth** in vitro
1943	Wilton Earle		Established L-cell mouse fibroblast cell line; first continuous cell line
1952	Renato Dulbecco		Use of **trypsin** for generation of replicate subcultures
1952	George Gey		Established first human cell line, HeLa, from cervical carcinoma
1955	Harry Eagle		Development of **defined media**
1977	Nelson-Rees & Flandermeyer		Confirmed HeLa cell cross-contamination of many cell lines
1998	Thompson et al		Culture of human embryonic stem cells

To Consider…

- **Pros**
  - Use of animals reduced
  - Homogenous cell population, same growth requirements
  - Control of the extracellular environment
  - Able to monitor various elements and secretions without interference from other biological molecules that occurs *in vivo*
- **Cons**
  - Remove interaction with other cells, hormones, support structures that would be present in vitro
  - Impossible to re-create *in vivo* environment. Artificial conditions could cause cells to de-differentiate or change phenotype

Applications

- Model systems
- Toxicity Testing
- Cancer Research
- Virology
- Cell-based Manufacturing
- Genetic Counselling
- Genetic Engineering
- Gene Therapy
- Drug Screening & Development

Primary Cultures & Continuous Cultures

- Primary Cultures (e.g. HUVEC, smooth muscle cells)
  - Enzymatically isolated from tissue
  - Finite lifespan
- Continuous/Immortalised Cell lines (e.g. HeLa)
  - Random mutation or deliberate modification
  - Indefinite proliferation

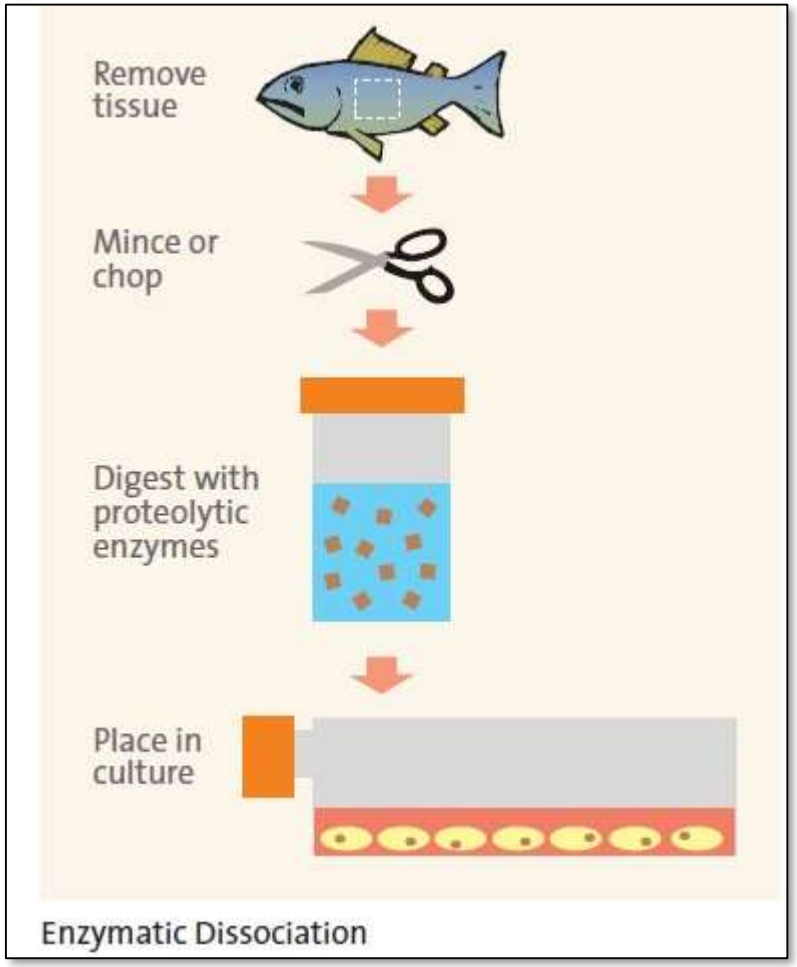
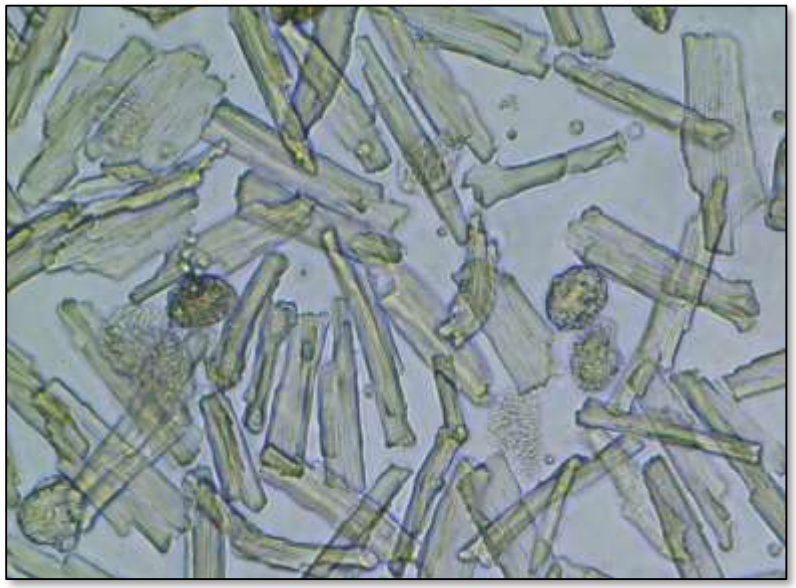


Image from 'Introduction to animal cell culture Technical Bulletin' Corning

Sources

- Freshly isolated (e.g. Hayley's rat cardiomyocytes)
  - Short term or medium term culture
  - Time consuming
  - Ethical approval



Rat ventricular cardiomyocytes –Hayley Crumbie

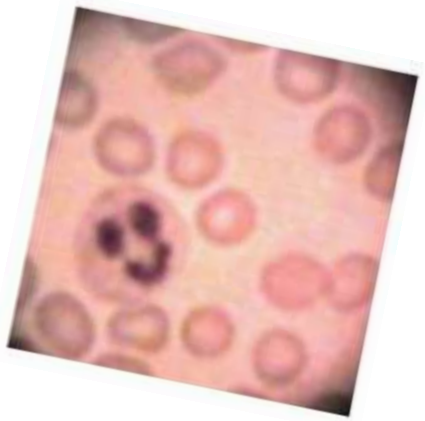
Sources

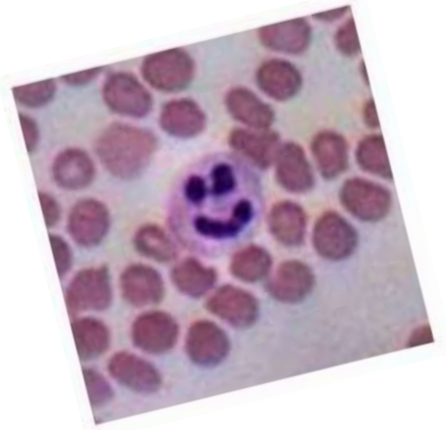
- Commercial
  - Sources include ATCC/ECACC, Life Technologies, Lonza, Cellworks,
  - Expensive
  - Screened, tested to ensure authenticity

- Other laboratories/co-workers
  - Questionable age, health and authenticity

The Science of Happy Cells

- More than just keeping them alive
- Increase in cell number
- Physiological and biochemical functions
- Optimum growth conditions
  - Cell specific
    - Temperature
    - Substrate for attachment
    - Medium
- Avoid Contamination





You never know what you might see down the microscope!

Growth Medium

- Provides nutrients
- Maintains pH & osmolality
- Components of basal media:
  - Salts
  - Carbohydrates
  - Vitamins
  - Amino Acids
  - Metabolic Precursors (e.g. sodium pyruvate)
  - Growth Factors
  - Hormones
  - Trace Elements

Requirements differ for cell types

Growth Medium

- Buffering system
  - CO_2/Sodium bicarbonate
  - Phosphate
  - HEPES
- Phenol Red (pH Indicator)
- L-Glutamine, GlutaMAX™
- Supplements
  - Antibiotics and Antimycotics
  - Serum

Warm before use!

Animal Sera

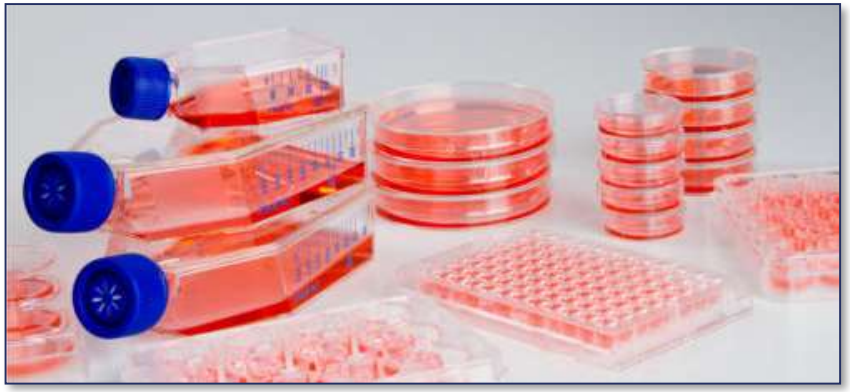
- Foetal and calf bovine serum common
- Rich source of:
  - Amino acids
  - Proteins
  - Vitamins (esp fat-soluble)
  - Carbohydrates
  - Lipids
  - Hormones
  - Growth factors
  - Minerals
  - Trace elements
- Batch variance

Freeze aliquots!

Low Serum or Serum-free cultures

- More common with the development of recombinant growth factors
- Allow more defined medium
- Allows optimisation for specific cell types

Growth Vessel

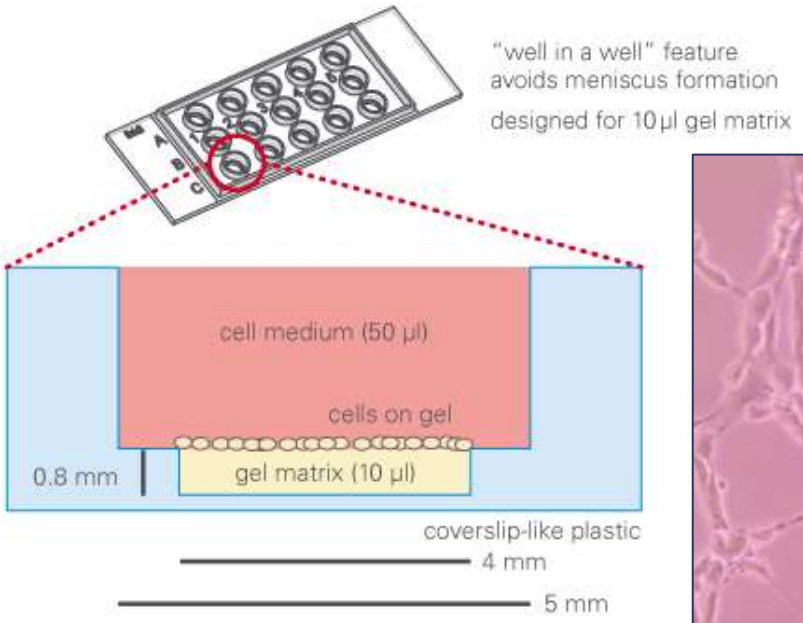


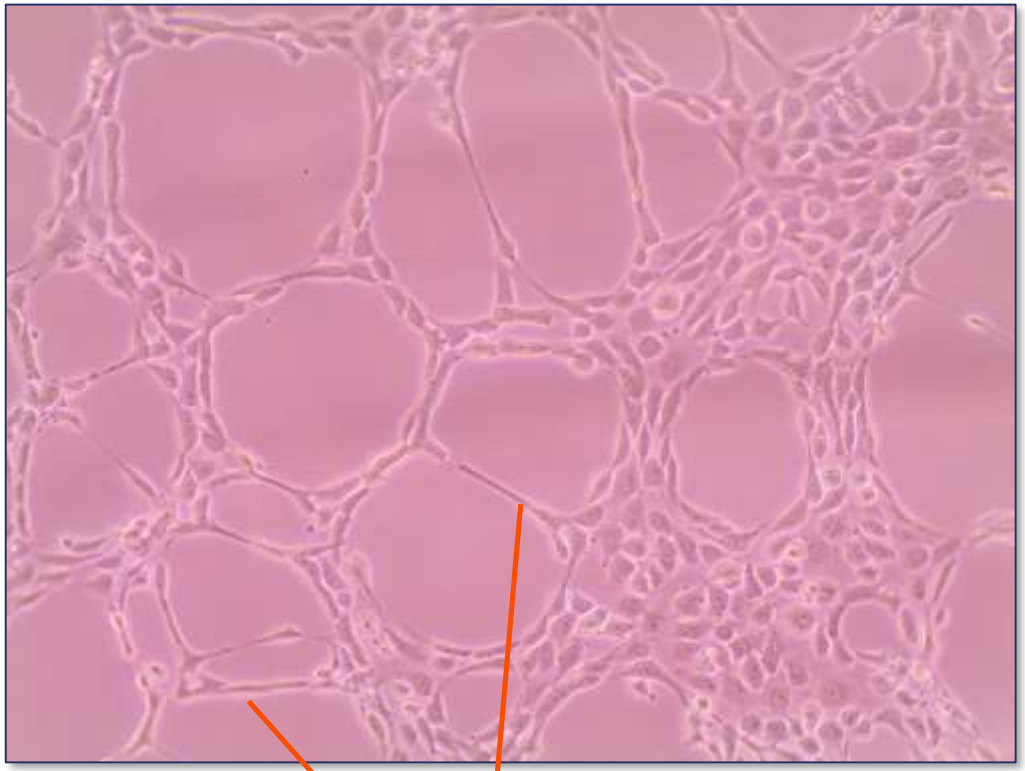
- Protection from contamination
- Substrate for attachment
- Polystyrene
  - Disposable
  - Better optical properties than glass
  - 'Tissue culture-treated' hydrophilic surface for better cell attachment

Alexis Carrel's first Pyrex D-Flask (1920's)

- Some cells require further treatment to attach, e.g. serum, collagen, laminin, gelatin, poly-l-lysine or fibronectin
- Some cells require 'Feeder cells' layers
- Specialised coatings e.g. Matrigel

Matrigel Endothelial Cell Tubule Assay





'Tubules'

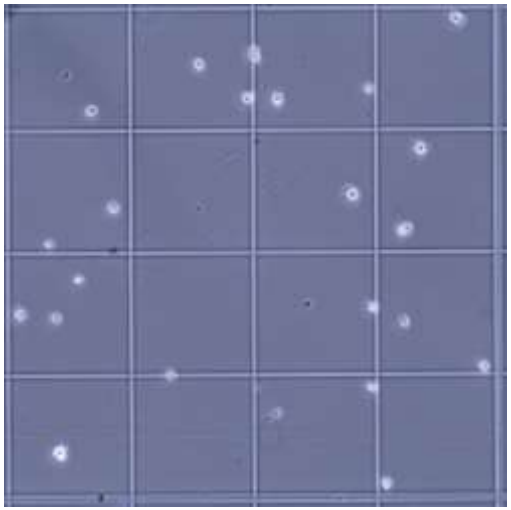
- Cells are grown on a gel matrix in small wells. Within a few hours, the cells form a network of inter-connecting branches, which can be seen under the microscope.
- The amount of branching and tubule formation is accepted as a measure of angiogenic potential.

Growth Conditions

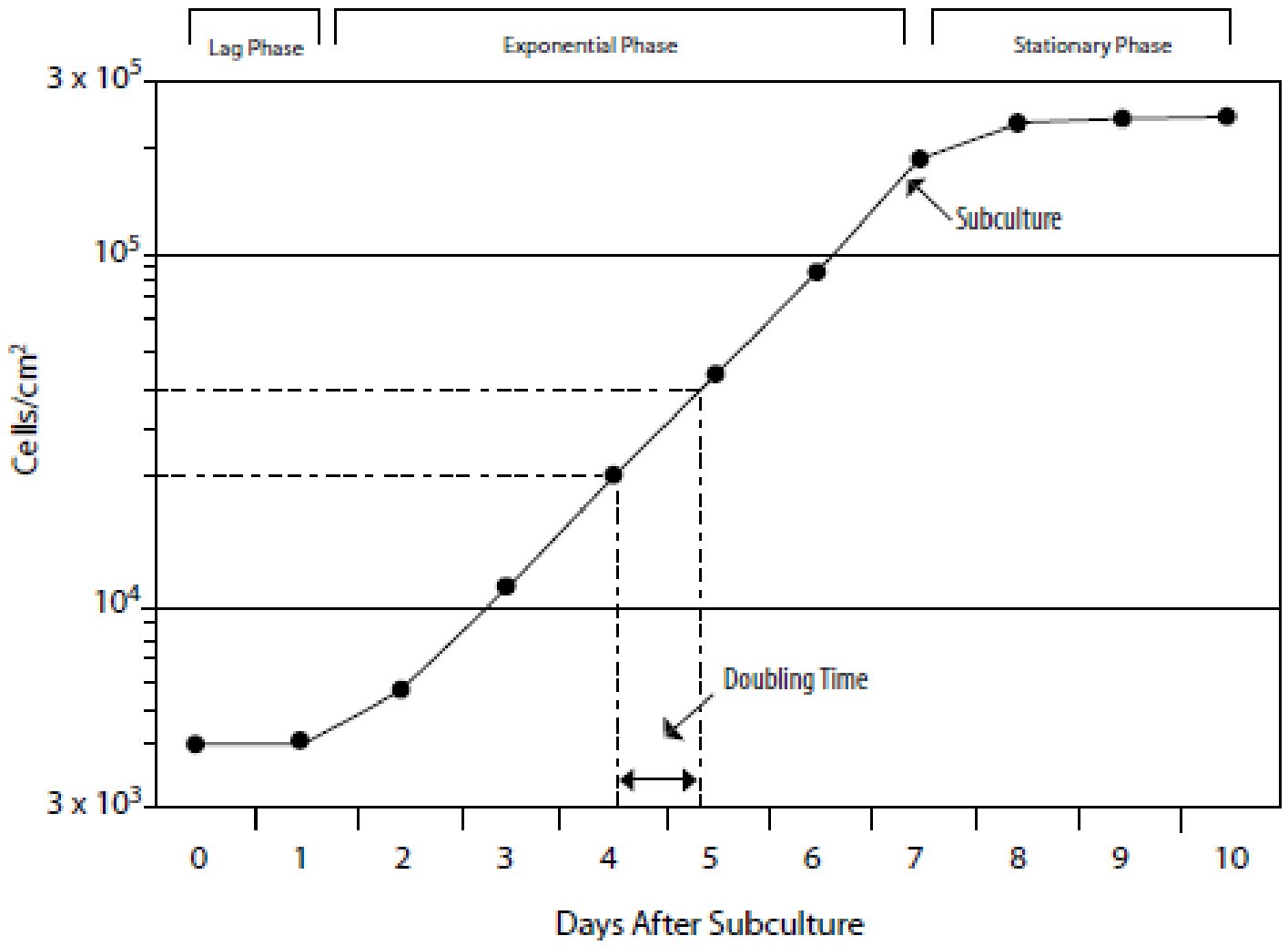
- **37°C** Body temperature
- **20% O_2** Optimal respiration
- **5% CO_2** Works with bicarbonate buffer to maintain pH of medium
- **Humidification** – Helps prevent evaporation of culture media from flasks, which would result in an increase in osmotic pressure - stresses or damages cells.

Are your cells happy?

- Morphology – phase contrast microscopy
- Expression of specialised functions or markers
- Cell number, growth rate, viability
  - Haemocytometer
  - Trypan blue



Cell Growth Curve (Image from ATCC Handbook)



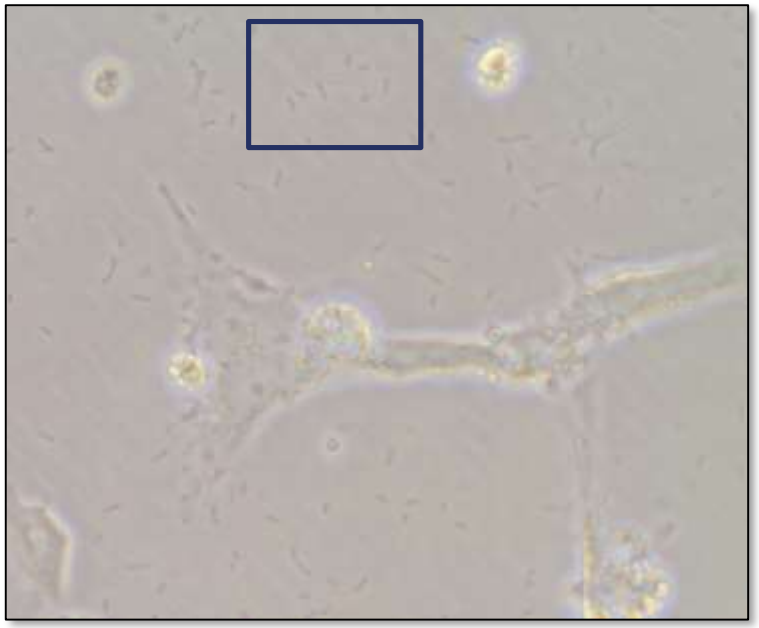
Subculturing

- Suspension cells
- Monolayer/Adherent cells
  - Require breakage of intercellular and intracellular cell-to-surface bonds
  - Mechanical dissociation (Cell Scraper)
  - Proteolytic dissociation
    - Trypsin/EDTA
- Passage number

Freezing

- Purpose is to ‘bank’ stocks of cells at low passage for future use.
- Use cryovials
- Cryoprotectant
  - DMSO
  - Glycerol
- Optimal freezing
  - ‘Mr Frosty’/Cool Cell 1°C/min in -80°C
  - Transfer to liquid nitrogen
- Thawing

Contamination



- Microbial Contamination
  - Bacteria, yeast, fungus, viruses, mycoplasma
  - Laminar Flow Hoods
  - Aseptic technique
  - Antibiotics
  - No **singing**!
- Chemical Contamination
- Cross-contamination with other cell lines

Department of Cardiovascular Sciences

- Facilities both at Glenfield and LRI (RKCSB)
- See **Martha Hardy** or **Julie Chamberlain** for an induction before using TC facilities, even if you have done cell culture elsewhere.
- This is **not** optional!

Further Reading

- Two excellent resources for protocols and authenticated cells
  - European Collection of Animal Cell Cultures (ECACC), a Public Health England Collection
    - www.pheculturecollections.org.uk/collections/ecacc.aspx
  - American Type Culture Collection (ATCC)
    - www.atcc.org.uk
- Corning Technical Bulletins
- 'The Immortal Life of Henrietta Lacks' Rebecca Skloot (2010),

ATCC®

ECACC
European Collection of Cell Cultures
A Health Protection Agency Culture Collection