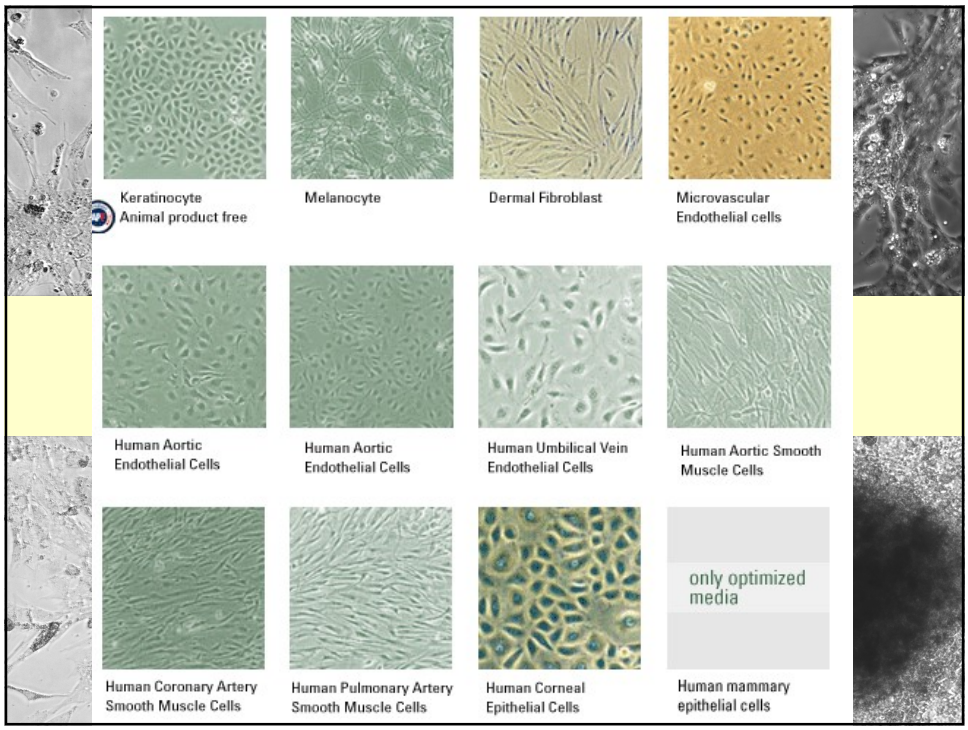
Cell biology
in vitro research

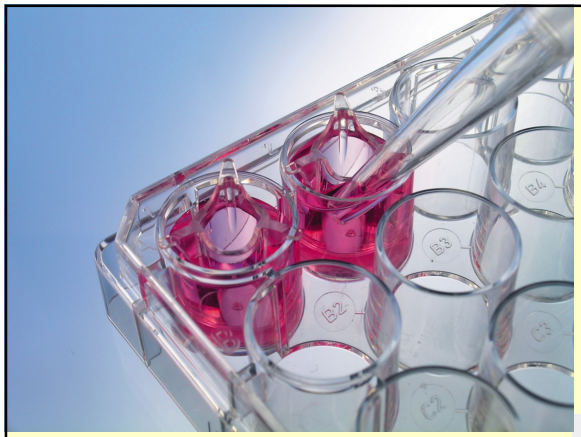
History

Haberlandt

Carrel

- 20th century
- Gottleib Haberlandt (plants) and Alexis Carrel (animals)

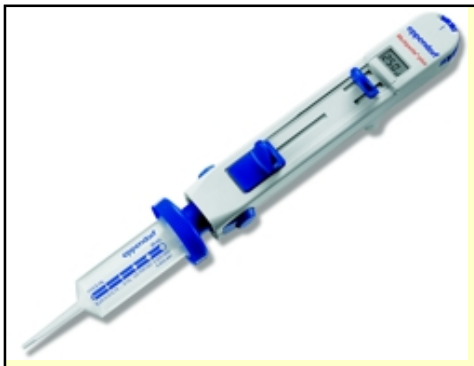

Keratinocyte
Animal product free
Melanocyte
Dermal Fibroblast
Microvascular
Endothelial cells
Human Aortic
Endothelial Cells
Human Aortic
Endothelial Cells
Human Umbilical Vein
Endothelial Cells
Human Aortic Smooth
Muscle Cells
Human Coronary Artery
Smooth Muscle Cells
Human Pulmonary Artery
Smooth Muscle Cells
Human Corneal
Epithelial Cells
only optimized
media
Human mammary
epithelial cells

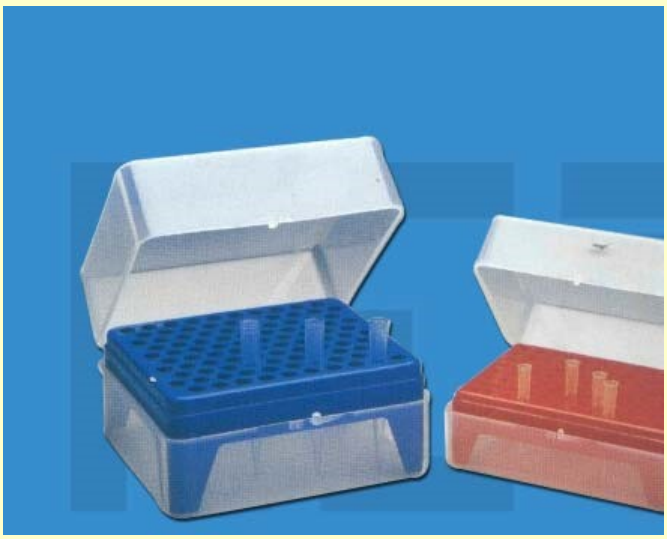


sterile conditions

Pipetting

Inverse *microscope*



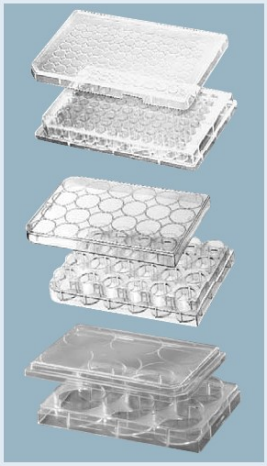


eppendorf
Centrifuge 5810 R

NEW

Cell⁺ TC dishes in *SURE*Grip Design

Order Number	Growth Area cm²	∅/Height mm	Packaging Bag/Case
83.1800.003*	8	35/10	20/500
83.1801.003*	21	60/15	20/500
83.1802.003*	58	100/20	20/500

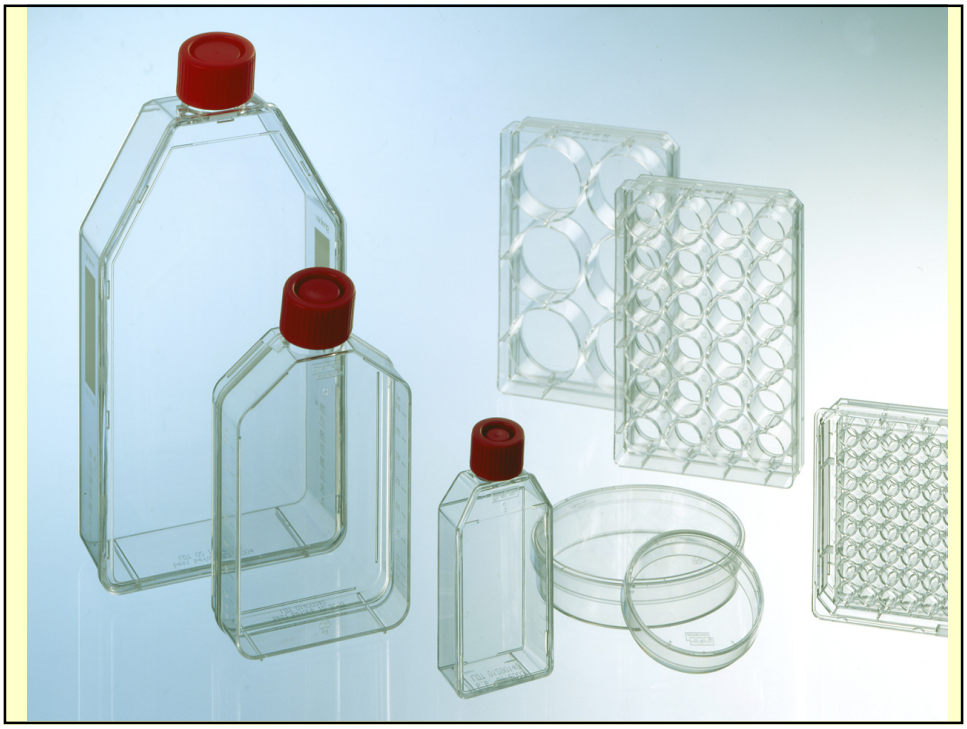


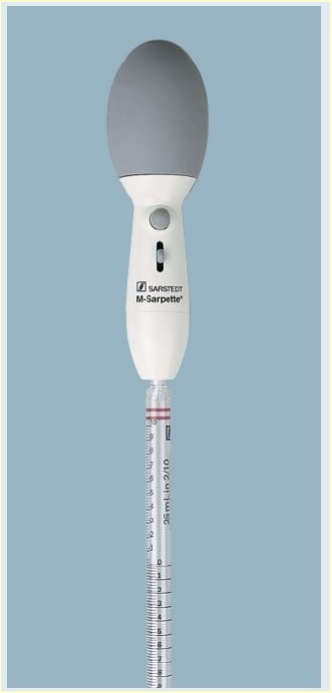
Cell⁺ TC plates

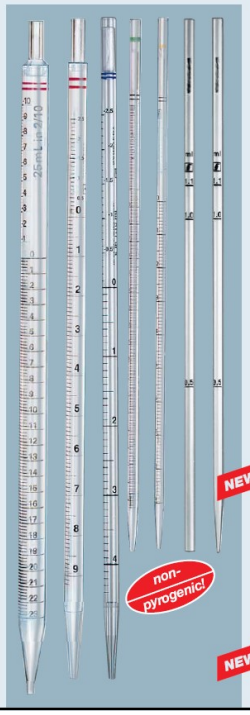
Order Number	Growth Area cm²	Max. Volume per Well	Number of Wells	Base Shape	Packaging Bag/Case
83.1835.300	0.35	0.38 ml	96	⌴	1/100
83.1836.300	2.0	3.6 ml	24	⌴	1/100
83.1839.300	9.5	17.2 ml	6	⌴	1/100

* *SURE*Grip Design

- Convenient handling due to a raised ring around the circumference of the dish, enabling an easy and secure grip of both the dish and lid, also from a whole stack.







Serological pipettes from clear polystyrene

- Non-pyrogenic, individually wrapped, sterile
- Easy identification by international colour code
- Larger pipetting volume due to negative graduation
- Variable mode of handling due to reverse graduation
- Optimised mouthpieces for universal fit in most common pipettors
- Easy opening, static-free packaging

Serological Pipettes 1 ml, 2 ml, 5 ml, 10 ml, 25 ml, 50 ml

Order Number	Total Volume/ Graduation	Description/ Colour Code	Packaging Pcs./Case
86.1251.001*	1 ml / 1/100 ml	plugged, ind. wrapped, sterile, yellow	1,000
86.1251.025	1 ml / 1/100 ml	plugged, sterile, 25 pcs., yellow	1,000
86.1252.001*	2 ml / 1/100 ml	plugged, ind. wrapped, sterile, green	1,000
86.1252.025	2 ml / 1/100 ml	plugged, sterile, 25 pcs., green	1,000
86.1253.001*	5 ml / 1/10 ml	plugged, ind. wrapped, sterile, blue	500
86.1253.025	5 ml / 1/10 ml	plugged, sterile, 25 pcs., blue	500
86.1254.001*	10 ml / 1/10 ml	plugged, ind. wrapped, sterile, orange	500
86.1254.025	10 ml / 1/10 ml	plugged, sterile, 25 pcs., orange	500
86.1685.001*	25 ml / 2/10 ml	plugged, ind. wrapped, sterile, red	200
86.1685.020	25 ml / 2/10 ml	plugged, sterile, 20 pcs., red	200
86.1689.001*	50 ml / 1/2 ml	plugged, ind. wrapped, sterile, violet	100

*non-pyrogenic

Aspiration Pipette, polystyrene

- For aspirating media with a vacuum pump
- Individually wrapped in paper/plastic peel packaging
- Certified non-pyrogenic
- Without graduation, without cotton plug

Order Number	Total Volume Graduation	Description	Packaging Pcs./Case
86.1252.011	2 ml/without graduation	without plug, without print, ind. wrapped, sterile	1,000

Dilution pipette with and without tip

Filtropur Filter Systems

Syringe filter Filtropur S for filtration of aqueous solutions up to 100 ml

- Bio-compatible prefilter is free from binding agents, and membrane is free from wetting agents
- Low protein binding membranes
- Individually wrapped and sterile
- Non-pyrogenic

Filtropur S 0.45 yellow housing for sterilisation and particle removal from solutions

Order Number	Inlet/Outlet Connection	Filtration Surface	Pore Size	Packaging Bag/Case
83.1826	inlet luer-lock female outlet luer-lock male	5.3 cm²	0.45 µm	1/50

Filtropur S 0.2 dark blue/neutral housing for sterile filtration

Order Number	Inlet/Outlet Connection	Filtration Surface	Pore Size	Packaging Bag/Case
83.1826.001	inlet luer-lock female outlet luer-lock male	5.3 cm²	0.2 µm	1/50

Filtropur S plus 0.2, blue/opaque housing with integral glass prefilter, for sterile filtration of viscous solutions

Order Number	Inlet/Outlet Connection	Filtration Surface	Pore Size	Packaging Bag/Case
83.1826.102	inlet luer-lock female	5.3 cm²	0.2 µm	1/50

Filtropur V vacuum filtration unit / Filtropur BT bottle top filter

- Improved performance for sterile filtration
- Low protein and nucleic acid binding
- Time and cost saving by higher throughput

Filtration units

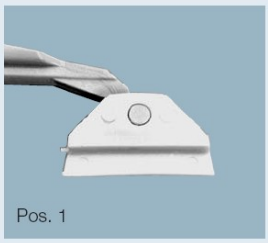
Order Number	Description	Filtration Volume	∅ Membrane	Pore Size	Packaging Bag/Case
83.1822	Filtropur V 25	250 ml	60 mm	0.45 µm	1/12
83.1822.001	Filtropur V 25	250 ml	60 mm	0.20 µm	1/12
83.1823	Filtropur V 50	500 ml	90 mm	0.45 µm	1/12
83.1823.001	Filtropur V 50	500 ml	90 mm	0.20 µm	1/12

A sterile screw cap for the collection vessel is included with each filtration unit.

Bottle top filter for storage vessel with an outer diameter of 45 mm* max.

Order Number	Description	Filtration Volume	∅ Membrane	Pore Size	Packaging Bag/Case
83.1822.100	Filtropur BT 25	250 ml	60 mm	0.45 µm	1/12
83.1822.101	Filtropur BT 25	250 ml	60 mm	0.20 µm	1/12
83.1823.100	Filtropur BT 50	500 ml	90 mm	0.45 µm	1/12
83.1823.101	Filtropur BT 50	500 ml	90 mm	0.20 µm	1/12

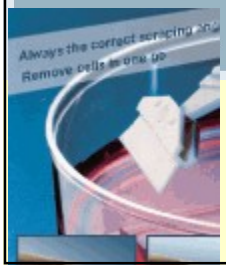
* Storage vessels with a thread diameter below 45 mm cannot be screwed onto the filter. However, a vacuum is nevertheless formed due to the special construction of the filter seal.



Pos. 1

NEW

NEW



Cell scraper with soft, flexible blade

- An alternative to enzymatic detachment of monolayer cultures to avoid cell alterations
- Easy and more complete separation of monolayer due to extra thin and flexible blade
- When downward pressure is applied to the handle, the flexible blade can remove all cells in its path with one smear
- Sterilised by gamma radiation, individually wrapped

Cell scraper with 2-Position Blade

Order Number	Cell Scraper Length cm	Blade mm	Packaging Bag/Case
83.1830	25	17	1/100
83.1832	16	13	1/100

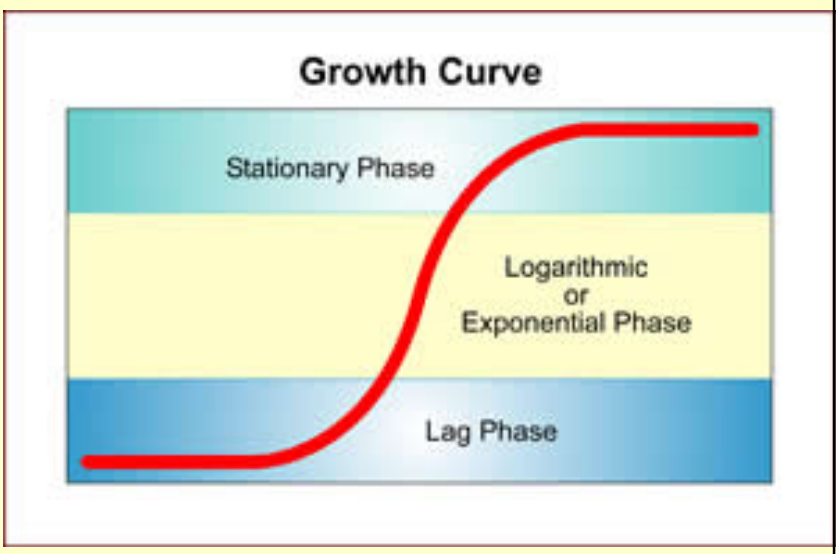
Cell scraper for use in larger TC flasks (T-175)

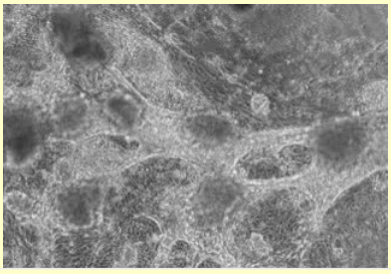
Order Number	Cell Scraper Length cm	Blade mm	Packaging Bag/Case
83.1831	39	31	1/100

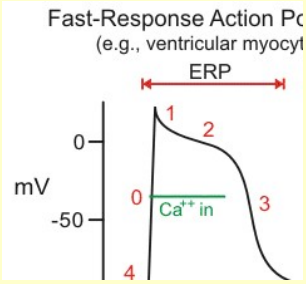
Why do we need sterile conditions when we culture animal or human cells.

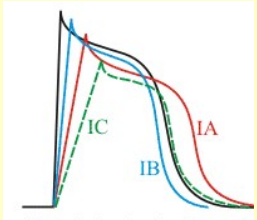
link

NEXT, we need tools for working with cells and influencing them









Ventricular Action Potentia

- Class IA: e.g., quinidine
  - Moderate Na^+-channel bl
  - ↑ ERP
- Class IB: e.g., lidocaine
  - Weak Na^+-channel blocka

study of the arrhytmia, effect on refractory stage

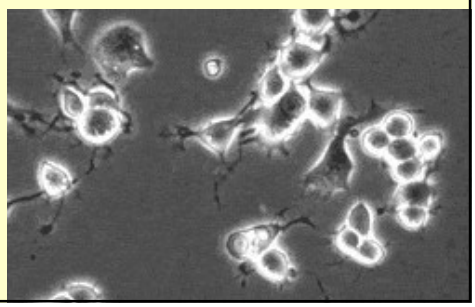
What do we need?

Cells, which we will cultivate

If we isolate it from the tissue, tools for dissociation

- **mechanical (scalpel, pipette)**
- **enzymatic (trypsin, collagenase)**
  - **optimal temperature**
  - **optimal time**

sterile plastics
medium
antibiotics
serum
growth factors



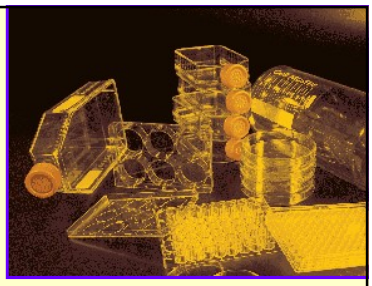
How to count cells before seeding

How to Change Cell Culture Media.mp4

What is *in vitro* cell culture approach good for according to you?

link

Cultivation conditions



- We use glass or plastics containers filled with medium and dispersed cells.
- The cells are incubated in the thermostat with controlled temperature, light and air composition.
- Cells will attach to the bottom of the container within hours and starts to divide.

Tissue culture products for adherent cells

PE ventilation cap

Non-wettable 0.2 µm hydrophobic filter membrane for reproducible gaseous exchange

Cell⁺ growth surface for sensitive cell cultures

Cell⁺ TC products for the growth of primary cultures and other fastidious cell lines are gamma-sterilised and made from high-quality polystyrene.

Cell⁺ TC products particularly support the growth of various fastidious cell lines:
- ☐ Primary cultures
- ☐ Continuous cell lines
- ☐ Cells that normally require a coating of colagen, gelatin or polylysine for growth
- ☐ Cell lines that grow poorly on standard surfaces

Cell⁺ TC flasks with quick-release cap
- ☐ Yellow cap with and without ventilation - Plug Seal Cap
- ☐ Two-position screw cap with locking mechanism
- ☐ Anti-tip skirt at canted neck

Order Number	Growth Area cm²	Volume ml	Screw Cap	Neck Shape	Packaging Bag/Case
83.1810.300	25	50	PE		20/500
83.1810.302 w. ventilation	25	50	PE	*	20/500
83.1813.300	75	250	PE		5/100
83.1813.302 w. ventilation	75	250	PE	*	5/100
83.1812.300	175	650	PE		5/40
83.1812.302 w. ventilation	175	650	PE	*	5/40

* with pouring channel · Replacement caps for TC flasks on p. 86.

Medium composition

- basic anorganic compounds
- trace elements
- stabilizers of pH (CO_3^{2-}/HCO_3^-, $HPO_4^{2-}/H_2PO_4^-$)
  - pH = 7,2 – 7,4
- glucose
- amino acids
- vitamins and other components of enzymes
- fatty acids and lipids
- specific proteins and peptides that support cell division
- serum (often foetal bovine serum)
- antibiotics (for lowering risk of bacteria contamination)

Media formulations	**DMEM**	**DMEM with stable glutamine**
	130-091-437	**130-091-438**
Components	g/L	g/L
Inorganic salts		
$CaCl_2 \cdot 2H_2O$	0.264	0.264
$Fe(NO_3)_3 \cdot 9H_2O$	0.001	0.001
KCl	0.4	0.4
$MgSO_4 \cdot 7H_2O$	0.2	0.2
NaCl	6.4	6.4
$NaHCO_3$	3.7	3.7
$NaH_2PO_4 \cdot H_2O$	0.125	0.125
Other components		
Glucose	4.5	4.5
Phenol red Na	0.0159	0.0159
Sodium Pyruvate	0.11	0.11
Amino acids		
L-Arginine.HCl	0.084	0.084
L-Cystine	0.048	0.048
L-Alanyl-L-Glutamine	--	0.868
Glycine	0.03	0.03
L-Histidine $HCl \cdot H_2O$	0.042	0.042
L-Isoleucine	0.105	0.105
L-Leucine	0.105	0.105
L-Lysine.HCL	0.146	0.146
L-Methionine	0.030	0.030
L-Phenylalanine	0.066	0.066
L-Serine	0.042	0.042
L-Threonine	0.095	0.095
L-Tryptophan	0.016	0.016
L-Tyrosine	0.072	0.072
L-Valine	0.094	0.094
Vitamins		
D-Ca Pantothenate	0.004	0.004
Choline Chloride	0.004	0.004
Folic acid	0.004	0.004
myo-Inositol	0.0072	0.0072
Nicotinamide	0.004	0.004
Pyridoxine.HCl	0.004	0.004
Riboflavin	0.0004	0.0004
Thiamine.HCl	0.004	0.004

Invitrogen offers several versions of DMEM including powder or liquid, with or without L-glutamine, High or Low Glucose, etc. The comparison table is a quick reference to help identify the catalog number that best suites your requirements:

Base Catalog Number	Glucose level	Liquid or Powder	With 4mM Glutamine	With GlutaMAX	With 1mM Sodium Pyruvate	With 25mM HEPES	Without Phenol Red	Without Glucose	Without Phosphate	Without Methionine and Cystine	Without Calcium
11965	4.5 g/L	Liquid	•								
11995	4.5 g/L	Liquid	•		•						
12430	4.5 g/L	Liquid	•			•					
21063	4.5 g/L	Liquid	•			•	•				
11971	4.5 g/L	Liquid	•						•		
12100	4.5 g/L	Powder	•								
12800	4.5 g/L	Powder	•		•						
11960	4.5 g/L	Liquid									
10313	4.5 g/L	Liquid			•						
31053	4.5 g/L	Liquid					•				
21013	4.5 g/L	Liquid								•	
21068	4.5 g/L	Liquid									•
10569	4.5 g/L	Liquid		•	•						
10564	4.5 g/L	Liquid		•		•	•				
10566	4.5 g/L	Liquid		•							
11885	1.0 g/L	Liquid	•		•						
11966	0.0 g/L	Liquid	•					•			
12320	1.0	Liquid	•		•	•					
31600	1.0	Powder	•		•						
11054	1.0	Liquid			•						
10567	1.0	Liquid			•						

NEXT, we need tolls for pipetting - pipettes, pipettors, micropipettes, tips in the sterile boxes

NEXT we needs instruments:

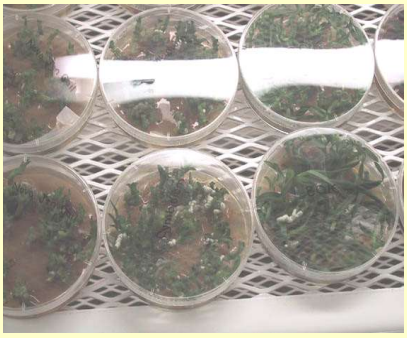
autoclave
incubator
sterile box

Cell biology
***in vitro* research II.**

objectives for which cell and tissue culture techniques have been developed

History

- first commercial use was related to plant micropropagation on artificial media of orchids, in 1920s
- Extensive research in this area took place in the 1950s and 1960s, but it was not until the creation of suitable artificial media (Murashige & Skoog, 1962) that plant micropropagation could be operated commercially.



cultured orchids

History

- a new stage in the cell cultures development is represented by genetic engineering and the possibilities of genetic modification of cells in the form of vectors and other tools.

What is necessary

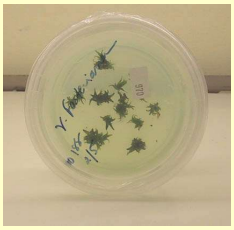
- suitable cells or tissues
- suitable cultivation technique
- sterile conditions (microorganisms can easily expel plant and animal cell)

- Growth regulatos– in plants, auxins & cytokine. U animals - lots of serum substance, there is an effort for identification and substitution
- Restoration medium condition and cells density must be adjusted so that there are not too many metabolites and still enough nutrients in the culture.

Micropropagation of plants

Micropropagation of plants

- Planting the explant into culture in a suitable medium. The medium contains substances that promote cell division, and can be liquid or solid.
- Each plant species has its own requirements for the medium and growth factors, which must be tested by trial and error.



Micropropagation of plants

- Cultivation in a suitable medium produces callus
- Cells of callus may can divide many times

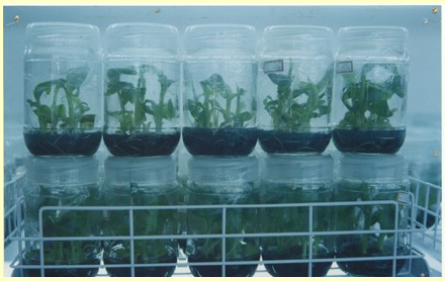
Setting up the optimal temperature and lightning condition is crucial during cultivation

Callus

- Callus is a cluster of undifferentiated plant cells. Undifferentiated cells have all cell walls of the same size, have a large nucleus and are free of vacuoles or have only a very small vacuole. Cells do not have any specialized structure or function. In nature, callus is formed in the plant wounds.
- *in vitro* is cell differentiation repressed by adding the growth regulators into the cultivation medium

Micropropagation of plants

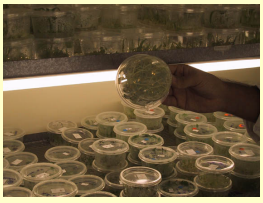
- Root formation - medium higher auxin:cytokine ratio



Young banana trees

Micropropagation of plants

- Planting plants, gradual decrease of humidity



Why is it convenient?

- One plant can by multiply many times
- The mother plant does not have to be destroyed
- Continuous supply of plants throughout the year

Animal cell cultures

- From tissue, tissue bank or stable cell line
- medium, aseptic conditions

culture vessel with medium and cells

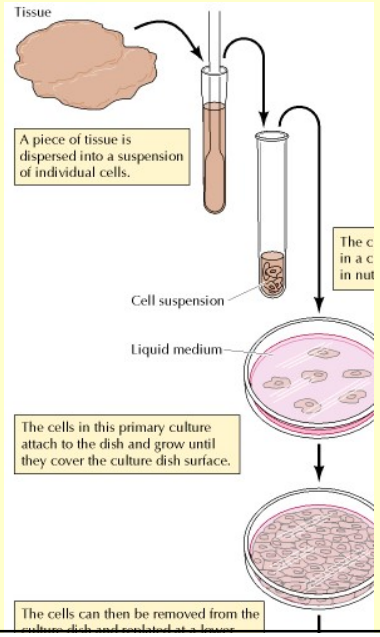
Animal cell cultures

- Optimal temperature, medium replacement
- Specific human cells for example need 37°C and 95% O_2, 5% CO_2

Cell passage

- After some time, the nutrients from the medium are depleted and the number of cells rises to an unbearable level.
- Therefore, it is necessary to remove a part of the cells, mix them with new medium and transfer this suspension to another culture vessel.

Cell culture



In terms of viability, we divide cell cultures into 3 groups:

- **Primary cultures**
  - contain cells freshly isolated from the organism
  - only those cells that are better adapted to the given conditions survive in the culture medium.
  - They exist usually only a few days, then the cells that have multiplied must be placed in new medium

In terms of viability, we divide cell cultures into 3 groups:

- **Cell lines**
  - based on primary cultures
  - cultures of normal diploid cells which was passaged at least one time
  - they arise from cells that have begun to proliferate *in vitro*
  - they disappear after about 40-50 divisions.

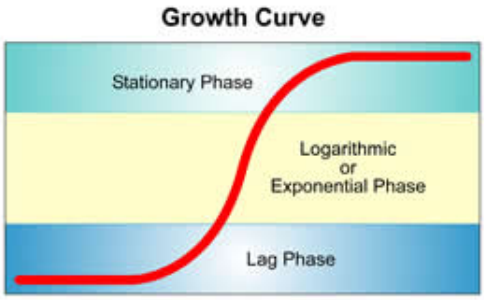
In terms of viability, we divide cell cultures into 3 groups:

- **Cell lines** (permanent cell lines)
  - they have character of tumor cells
  - they are fully adapted to the *in vitro* conditions
  - they can divide indefinitely and can be maintained for any length of time
  - they are created by the transformation of cell lines or are cells originally isolated from tumours

Cell culture growth curve

- expresses changes in cell number over time
- Cell culture growth has the following phases:
- **Lag-phase** – initial phase, the number of cells first decreases slightly and then increases relatively quickly. The cells are adapted to the culture medium and prepared for cell division.

Log-phase (logarithmic or exponential phase) - the number of cells grows exponentially. At this stage, we capture the most cells in mitosis, which can be used to observe chromosomes.

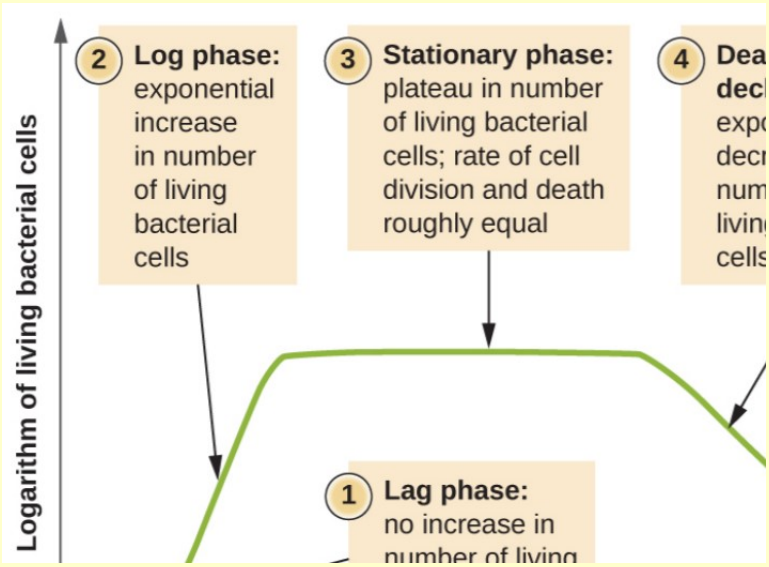


Growth Pattern of Cultured Cells

– **Stationary phase** – growth of cells decreases, inhibitory effects (like contact inhibition, inhibitors of growth) take place the growth medium is spent (i.e., one or more of the nutrients is depleted) or when the cells occupy all of the available substrate

– **Death phase -** the cell number decreases – cells are dying because of insufficient nutrients amount, inappropriate pH and increase in toxic compounds coming from metabolism

Growth Pattern of Cultured Cells



differences between animal and plant cell cultures

- animal cells possess limited number of cell cycles
- animal cells require more intensive care
- culture media are less defined in term of particular compounds composition

differences between animal and plant cell cultures

- biohazard

Benefits of cell cultures

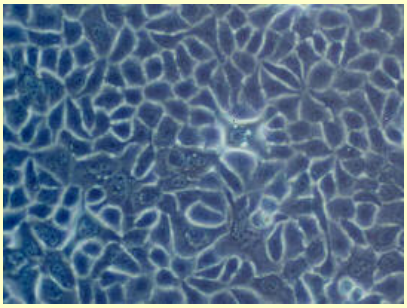
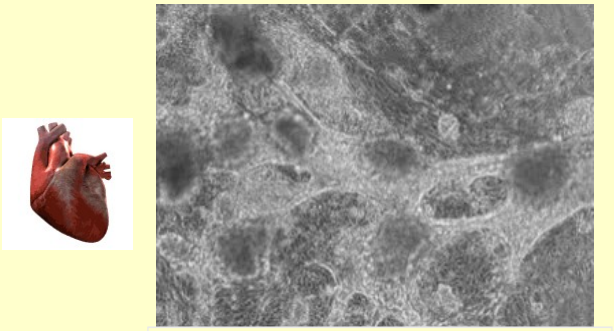
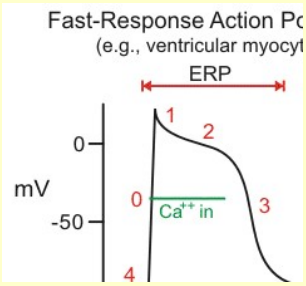
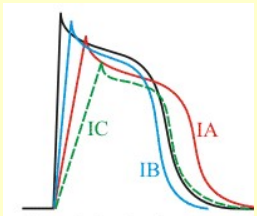


Photo courtesy of Sigma Aldrich

- to growth viruses that need host cells
- generation of monoclonal antibodies for research and diagnostics
- research
- genetic manipulations







Ventricular Action Potentia

- Class IA: e.g., quinidine
  - Moderate Na^+-channel bl
  - ↑ ERP
- Class IB: e.g., lidocaine
  - Weak Na^+-channel blocka

arrhythmia research, effect of refractory stage

- in basic research experiments usually both approaches, *in vitro* as well as *in vivo*, can be used
- *in vitro* approach allows more detailed study of cell functions but does not reflect system regulations
- therefore, it is advisable to combine both, *in vitro* as well as *in vivo* approaches

Thank you for your attention