Corso di biologia della cellula e dei tessuti 2009-2010

Introduzione alle colture cellulari

Silvia De Marchis

Perché coltivare le cellule????

Ridurre la complessità tissutale:

studiare la biologia delle cellule (metabolismo, differenziamento, proliferazione, migrazione) VIDEO1 – VIDEO2- VIDEO3

Manipolare l'ambiente:

studiare l'effetto di vari composti chimici su tipi cellulari differenti

Modificare geneticamente le cellule:

sintesi su larga scala di proteine terapeutiche/diagnostiche, produzione di vaccini, sviluppo di approcci di terapia genica

Produrre tessuti artificiali:

studiare la combinazione di tipi cellulari e fattori molecolari per generare tessuti artificiali es. pelle, cornea, congiuntiva per trapianti

Colture di cellule, tessuti, organi…

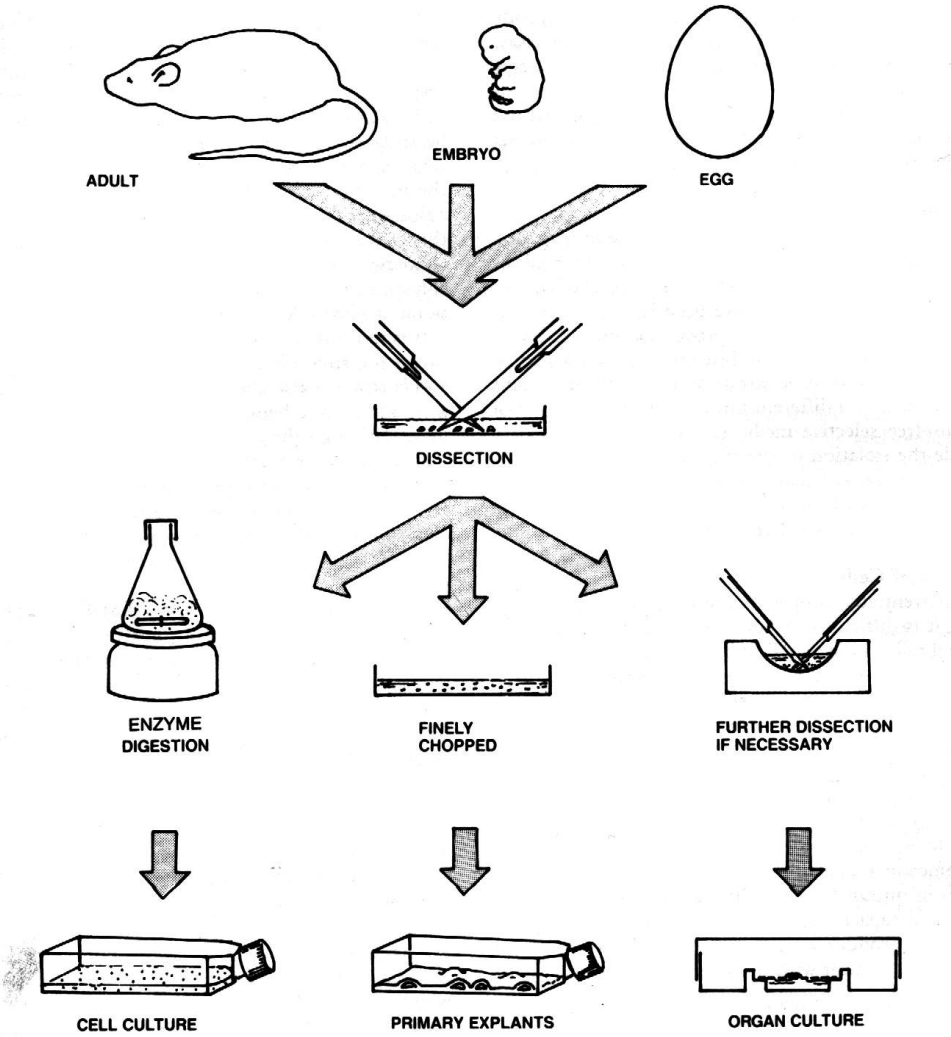


Fig. 1.2. Types of tissue culture.

I pionieri della tecnica di coltura

1882-1885 -Sydney Ringer mette a punto la composizione di una soluzione salina (Soluzione Ringer) efficace nel mantenere battito di cuore isolato dal corpo.

1885 -Wilhelm Roux mantiene in vita per alcuni giorni una porzione di tessuto neurale derivato dell'embrione di pollo, mantenendolo in una soluzione salina a T controllata

1907- Ross Harrison mantiene in coltura tessuto neurale di anfibio in un coagulo di linfa - dimostra origine assoni da singoli neuroni

1910-1923- Montrose Burrows e Alexis Carrel sviluppano un metodo di coltura per animali a sangue caldo (cane, gatto, pollo, ratto, cavia, tumori umani)
usano coagulo di plasma + Sali + siero. Sviluppano la prima linea cellulare

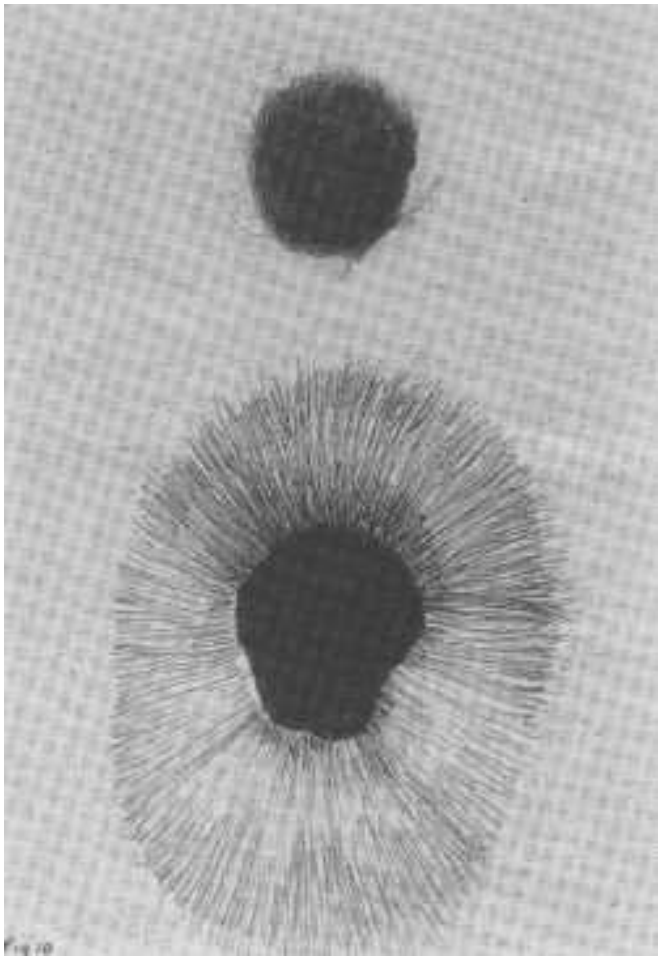
anni '40-'50- ampio sviluppo tecniche colture cellulari in supporto alla ricerca in ambito virologico-sviluppo vaccini es. vaccino anti-polio (Jonas Salk- introdotto in USA nel 1955)

1954 - Premio Nobel a Enders, Weller e Robbins per la scoperta del metodo di produzione del vaccino anti-polio utilizzando colture cellulari derivanti da rene di scimmia

1954 - Rita Levi-Montalcini coltiva gangli sensoriali di pollo e scopre il fattore neurotrofico NGF

Per saperne di più:
http://www.corning.com/lifesciences/us_canada/en/about_us/cell_culture_history.aspx

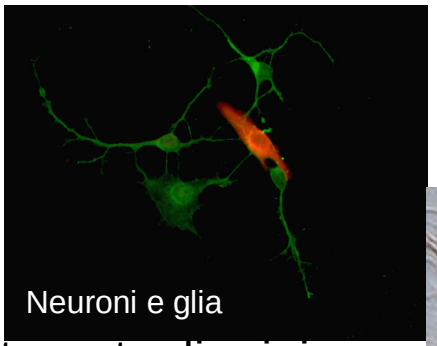
1986 Nobel Prize
Rita Levi- Montalcini & Stanley Cohen

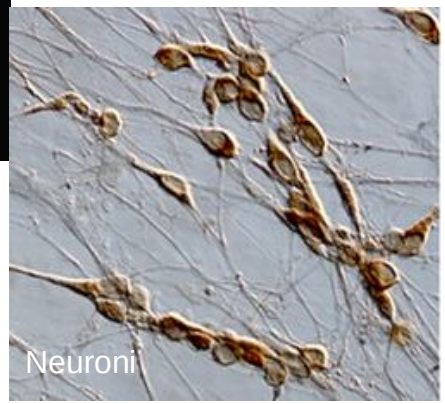


Nerve Growth Factor

Colture cellulari

Colture primarie





Isolate direttamente dal tessuto

Cellule eterogenee, simili alle cellule del tessuto di origine

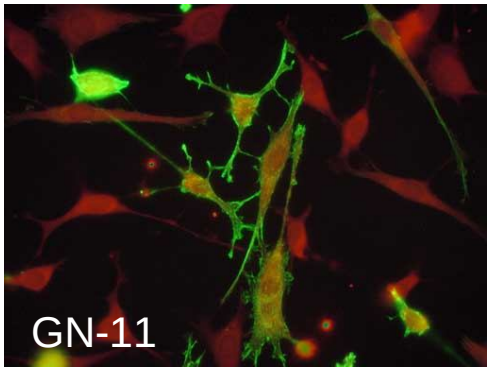
Limitate capacità di proliferazione -senescenza

Linee cellulari

Ottenute per sub-coltura da colture primarie, possono essere immortalizzate attraverso mutazioni spontanee o indotte

Modificazioni rispetto al fenotipo/genotipo originali

Possibilità di conservazione mediante congelamento in azoto liquido



ATCC ECACC DSMZ

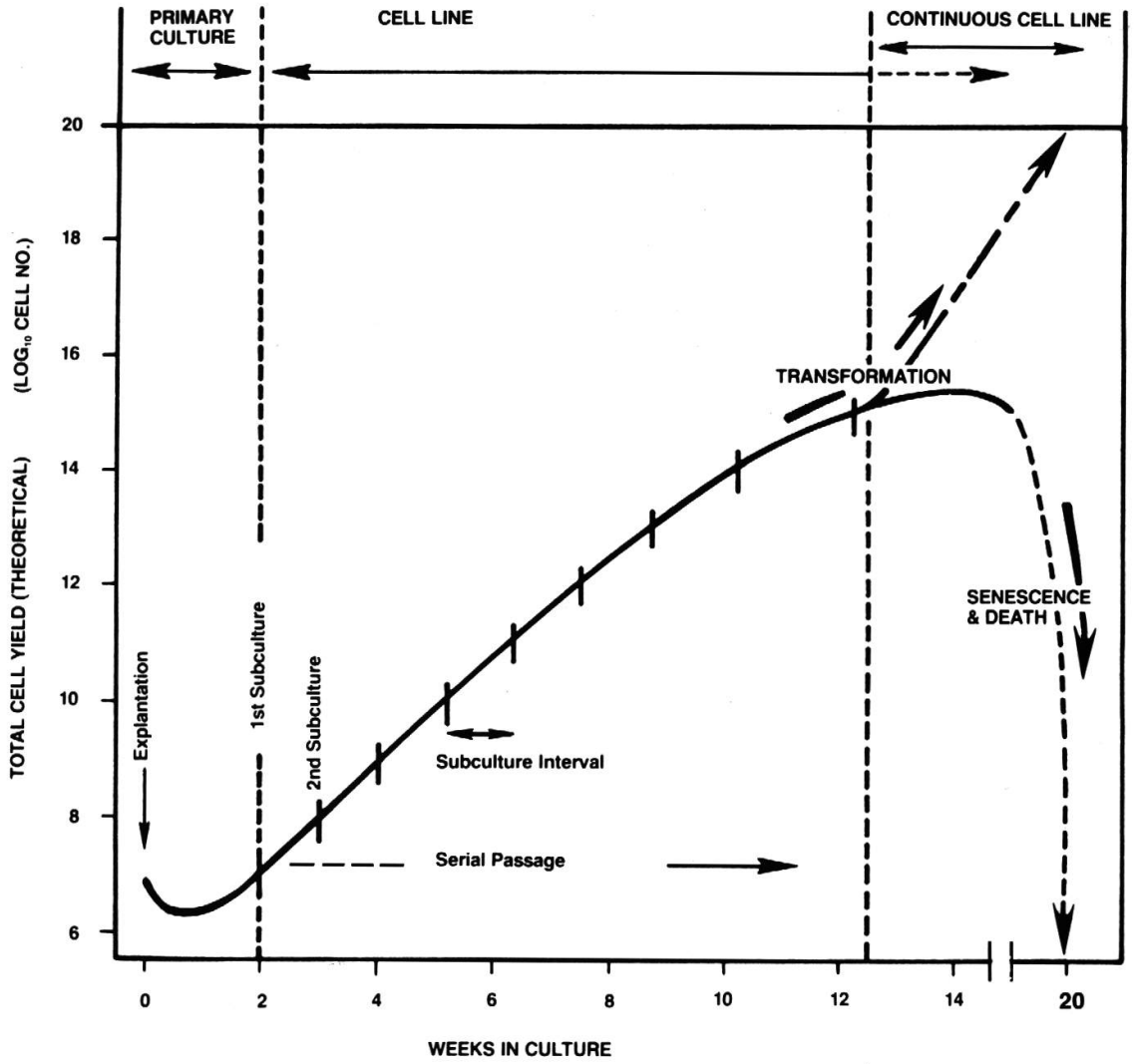


Fig. 2.1. Evolution of a cell line. The vertical axis represents total cell growth (assuming no reduction at passage) for a hypothetical cell culture. Total cell number (cell yield) is represented on the Y-axis on a log scale and time in culture, on the X-axis on a linear scale. Although a continuous cell line is depicted as arising at 12½ wk it could, with different cells, arise at any time. Likewise, senescence may occur at any time, but for human diploid fibroblasts it is most likely to occur between 30 and 60 cell doublings or 10 to 20 wk, depending on the doubling time. Terms and definitions used are as in the Glossary. Transformation is explained in more detail in Chapter 15.

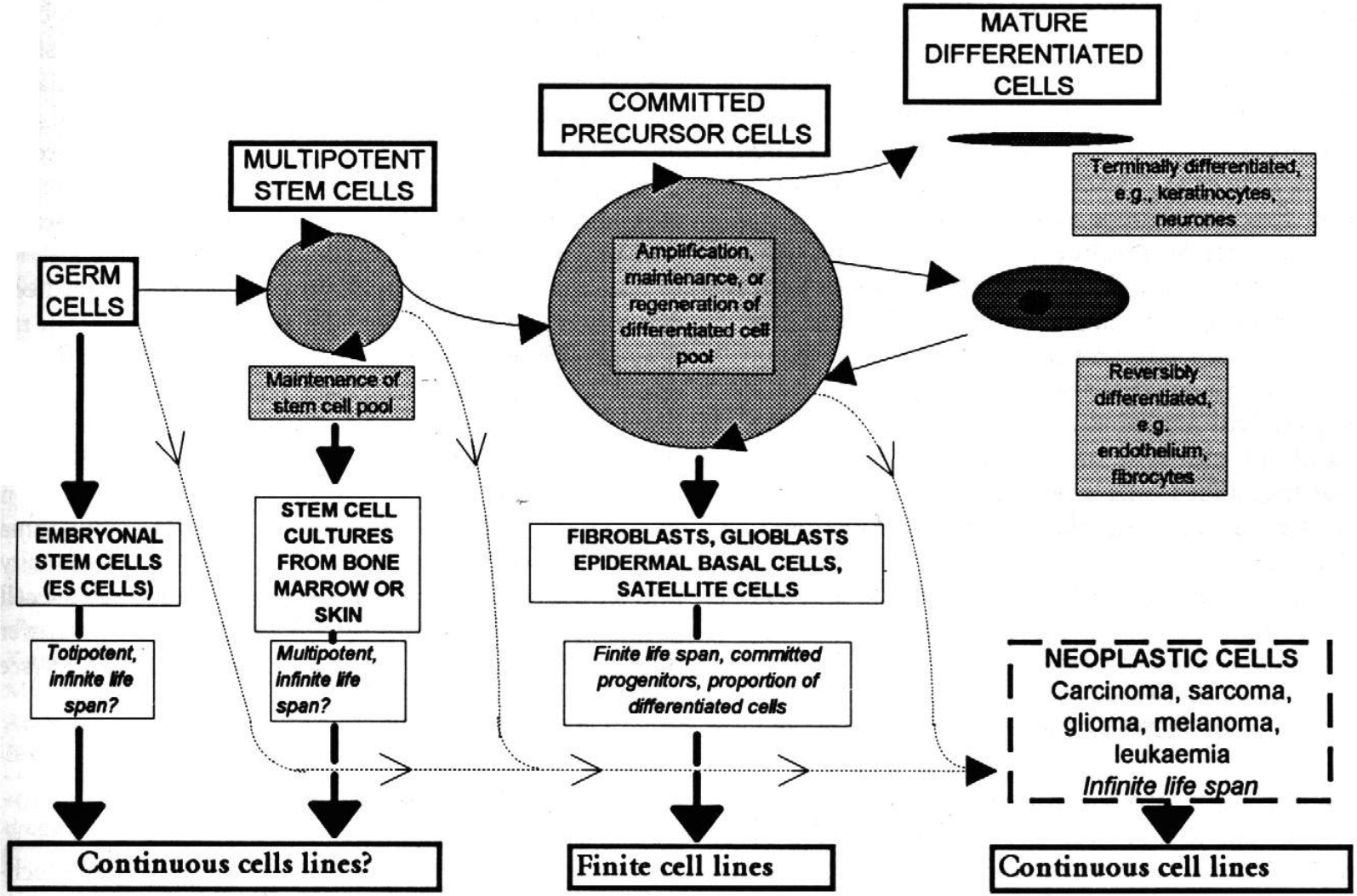


Fig. 2.3. *Origin of cell lines. With a few exceptions (e.g., differentiated tumor cells) culture conditions select for the proliferating progenitor cell compartment of the tissue or induce cells that are partially differentiated to revert to a progenitor status. While neoplastic cells, and cell lines, may be derived from differentiated cells, it seems more likely that they arise from malignant progenitor cells, some of which retain the capability to divide, while others continue to differentiate.*

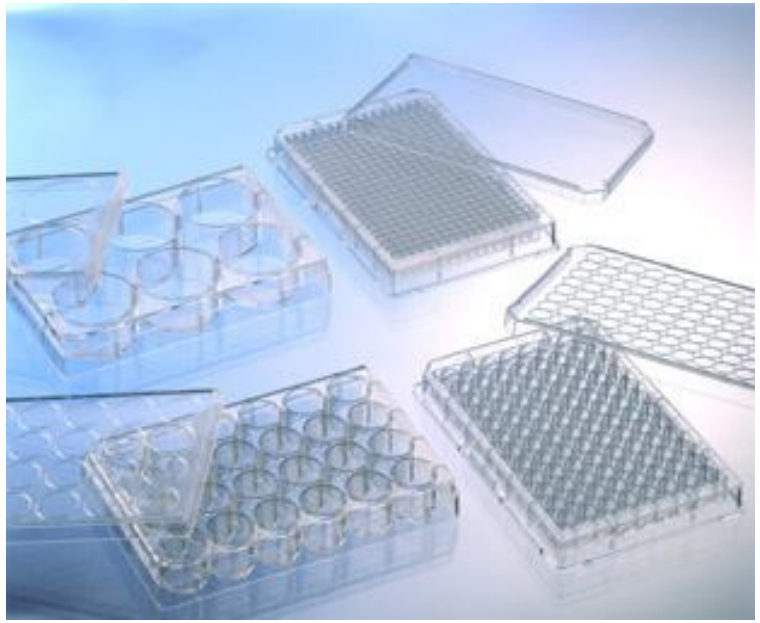
Come coltivare le cellule

Ambiente

- Temperatura
- Gas
- Terreni di coltura
- Substrati di adesione

Supporti per coltivare le cellule

Polistirene
Biologicamente inerti
Non tossici
Trasparenti



Parametri critici:
Temperatura, umidità e CO_2

Incubatore

Condizioni standard (mammifero)
T 37°C, 5% CO2,
umidità relativa (RH) >98%

Terreni base di coltura

http://www.sigmaaldrich.com/life-science/cell-culture/classical-media-salts.html

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Dulbecco's Modified Eagle's Medium (DME)

Many modifications of Eagle's Medium have been developed since the original formulation appeared in the literature. Among the most widely used of these modifications is Dulbecco's Modified Eagle's Medium (DME).

DME is a modification of Basal Medium Eagle (BME) that contains a 4-fold higher concentration of amino acids and vitamins, as well as additional supplementary components. The original DME formula, first reported for culturing embryonic mouse cells, contained 1,000 mg/L of glucose. An alteration with 4,500 mg/L glucose is optimal in cultivating certain cell types.

	D0422	D1145	D0819	D1152
	[1×]	[1×]	[1×]	[1×]
COMPONENT	g/L	g/L	g/L	g/L
Inorganic Salts				
$CaCl_2 \cdot 2H_2O$	0.2	0.2	0.2	0.2
$Fe(NO_3)_3 \cdot 9H_2O$	0.0001	0.0001	0.0001	0.0001
$MgSO_4$	0.09767	0.09767	0.09767	0.09767
KCl	0.4	0.4	0.4	0.4
$NaHCO_3$	3.7	3.7	3.7	—
NaCl	6.4	6.4	6.4	4.4
NaH_2PO_4	0.109	0.109	0.109	0.109
Amino Acids				
L-Alanyl-L-Glutamine	—	—	0.869	—
L-Arginine • HCl	0.084	0.084	0.084	0.084
L-Cysteine • 2HCl	—	0.0626	0.0626	0.0626
L-Glutamine	—	—	—	0.584
Glycine	0.03	0.03	0.03	0.03
L-Histidine • HCl • H_2O	0.042	0.042	0.042	0.042
L-Isoleucine	0.105	0.105	0.105	0.105
L-Leucine	0.105	0.105	0.105	0.105
L-Lysine • HCl	1.46	0.146	0.146	0.146
L-Methionine	—	0.03	0.03	0.03
L-Phenylalanine	0.066	0.066	0.066	0.066
L-Serine	0.042	0.042	0.042	0.042
L-Threonine	0.095	0.095	0.095	0.095
L-Tryptophan	0.016	0.016	0.016	0.016
L-Tyrosine • 2Na • $2H_2O$	0.10379	0.6351	0.10379	0.10379
L-Valine	0.094	0.094	0.094	0.094
Vitamins				
Choline Chloride	0.004	0.004	0.004	0.004
Folic Acid	0.004	0.004	0.004	0.004
myo-Inositol	0.0072	0.0072	0.072	0.0072
Niacinamide	0.004	0.004	0.004	0.004
D-Pantothenic Acid • ½Ca	0.004	0.004	0.004	0.004
Pyridoxal • HCl	—	—	—	0.004
Pyridoxine • HCl	0.004	0.004	0.004	—
Riboflavin	0.0004	0.0004	0.0004	0.0004
Thiamine • HCl	0.004	0.004	0.004	0.004
Other				
D-Glucose	4.5	4.5	4.5	4.5
HEPES	—	—	—	5.958
Phenol Red • Na	0.0159	—	0.0159	0.0159
Pyruvic Acid • Na	0.11	—	—	—
ADD				
Glucose	—	—	—	—
L-Glutamine	0.584	0.584	—	—
$NaHCO_3$	—	—	—	3.7

Terreni di coltura controllo pH

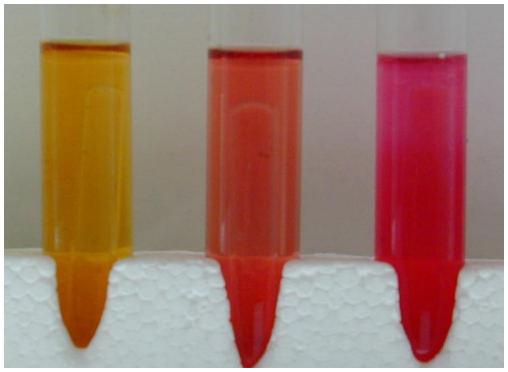
- pH 7,2-7,5
- Tampone bicarbonato ($NaHCO_3$)

$$HCO_3^- + H^+ + \leftrightharpoons H_2CO_3 \leftrightharpoons CO_2 + H_2O$$

acido ←

basico →

Rosso fenolo



pH<6,8 pH>7,4

Hepes (*4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid*)

Terreno di coltura completo

Terreno base (composizione definita)

+

Glutammina

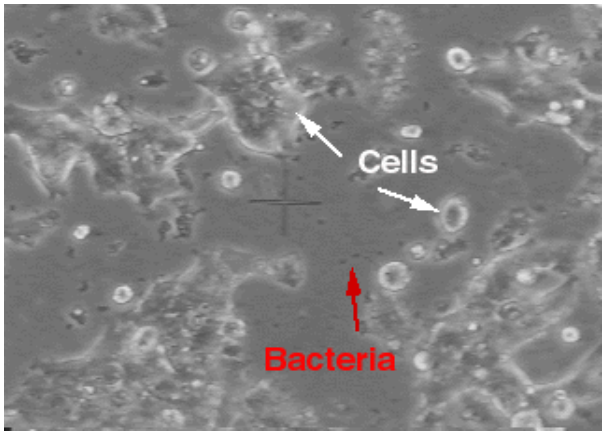
Siero (composizione non definita)

http://www.sigmaaldrich.com/life-science/cell-culture/sera.html

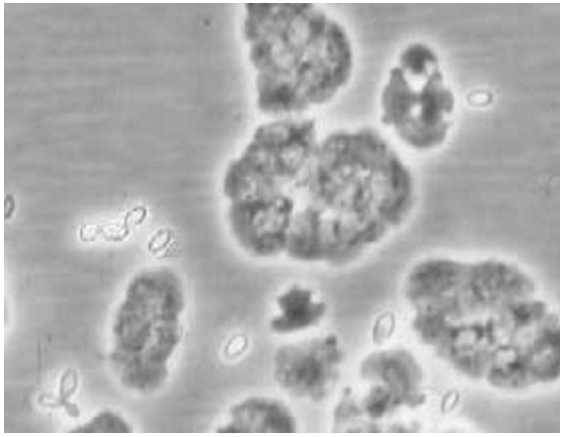
Antibiotici (penicillina/streptomicina)

Contaminazione

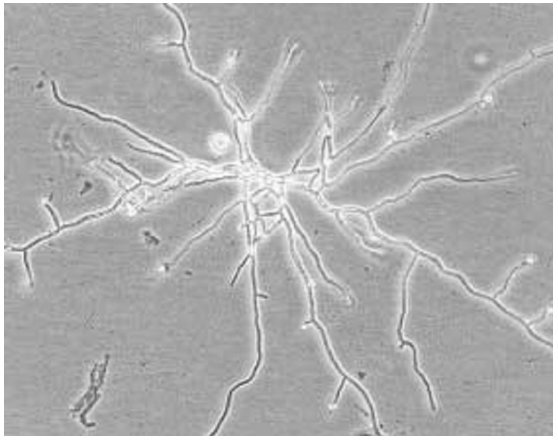
batteri



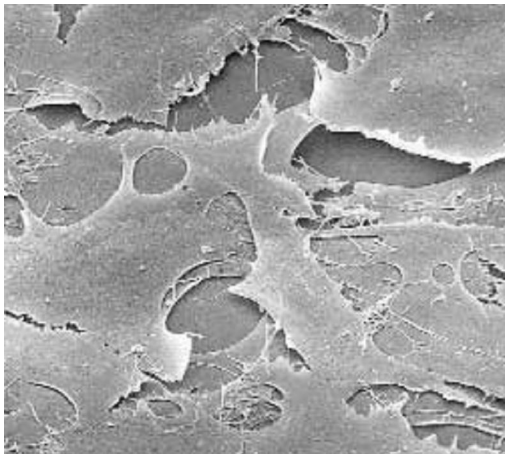
lieviti

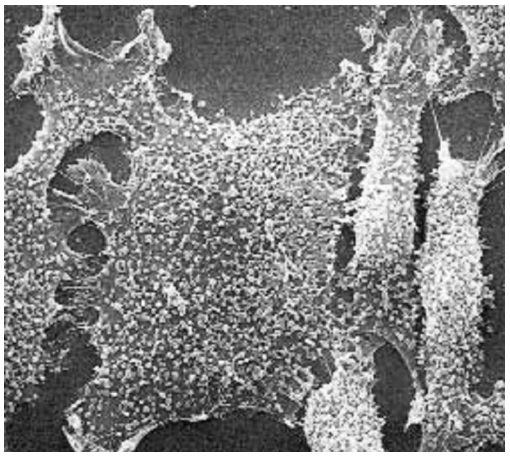


muffe



Micoplasmi (Non osservabili al microscopio rovesciato)





STERILITA’

Utilizzo di terreni, reagenti e plasticheria sterile

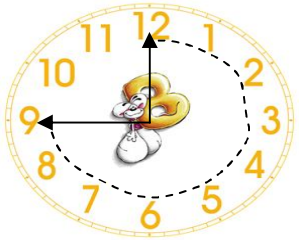
Manipolare le cellule in ambiente sterile

Substrati di adesione

Trattamento delle piastre:

++++++
Poly-D-Lysine (PDL)
Poly-L-Lysine (PLL)

Proteine della matrice extracellulare:
Collagene
Laminina
Gelatina
Fibronectina



Cosa faremo in laboratorio…

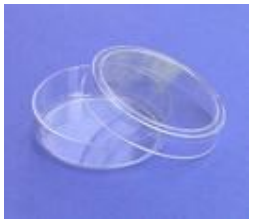
1. Com’è organizzato un laboratorio di colture cellulari?

2. Come appaiono al microscopio rovesciato cellule vive, adese alle base della piastra di coltura? Quali organuli della cellula sono riconoscibili?

4. Come posso mettere in evidenza parti della cellula che non sono apprezzabili al microscopio rovesciato basandomi sul solo contrasto? Marcatura dei microfilamenti di actina.

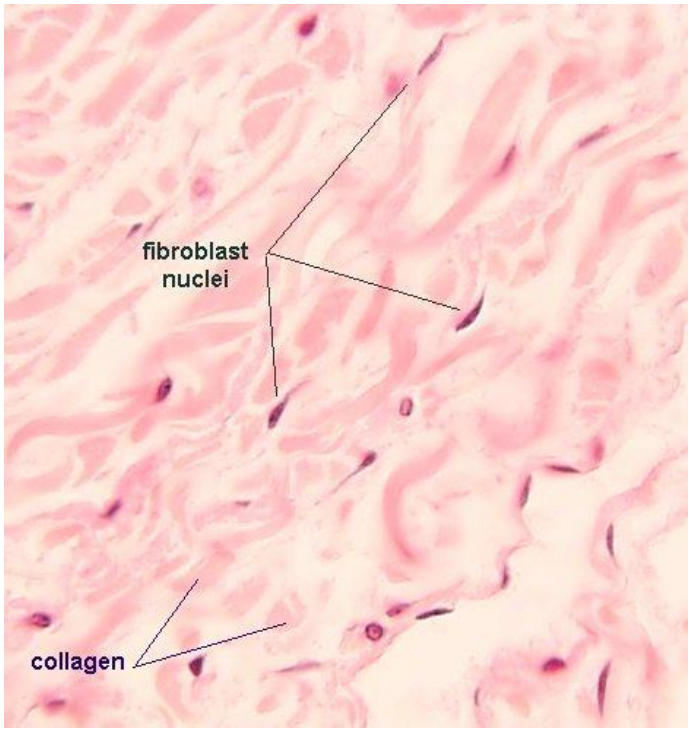
3. Come cambia la morfologia delle cellule quando le stacchiamo dalla piastra mediante trattamento enzimatico?

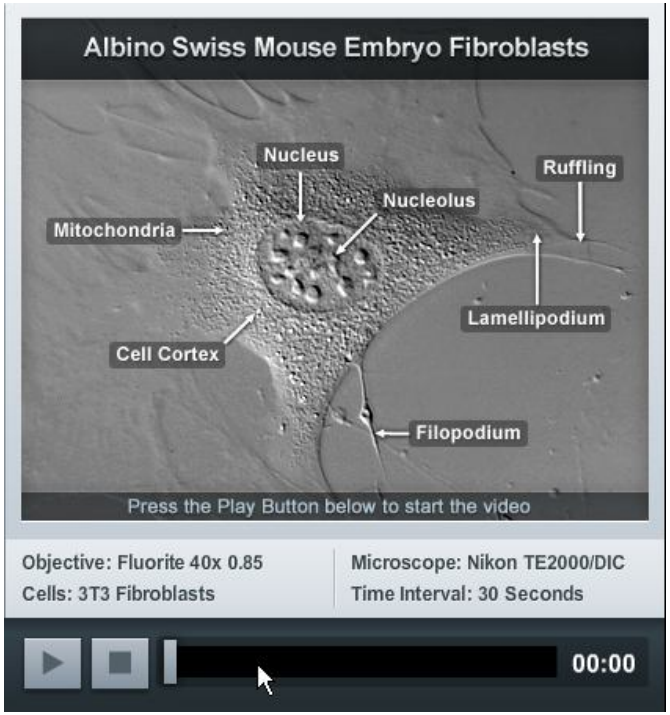
NIH 3T3 "3-day transfer, inoculum 3 x 10^5 cells per 20-cm^2dish"



I fibroblasti sono cellule tipiche del tessuto connettivo, in grado di produrre le componenti della matrice extracellulare.

Aderiscono alla plastica - non necessitano di substrati di adesione particolari





Todaro & Green 1962
Dept. Pathology NY University

Distacco delle cellule dal substrato

Protocollo:

1. Aspirare il terreno dalle piastre
2. Lavare le cellule con PBS senza Ca2+ e Mg2+
3. Incubare le cellule in presenza di tripsina/EDTA (37°C per 2’ o T° amb per 5 min circa)
4. Osservare le cellule al microscopio
5. Favorire il distacco mediante agitazione

Marcatura dei microfilamenti di actina

Protocollo:

1. Fissare le cellule 20’ in PFA4%
2. Lavaggio in PBS per 5’
3. Incubazione con Alexa Fluor® phalloidin* 1:50 per 10’
4. Osservazione al microscopio a fluorescenza

*La falloidina è una micotossina che lega i filamenti di actina inibendone la depolimerizzazione

CELL CULTURE GLOSSARY

Cell Culture – Establishment and maintenance of cultures derived from dispersed cells taken from original tissues, primary culture, or from a cell line or cell strain.

Cell Line – Immortalized cell, which have undergone transformation and can be passed indefinitely in culture.

Cell Clones – Individual cells separated from the population and allowed to grow.

Primary Culture – Cells resulting from the seeding of dissociated tissues, i.e. Huvec cells.

Cell Passage – The splitting (dilution) and subsequent redistribution of a monolayer or cell suspension into culture vessels containing fresh media.

Confluency – The confluency of a culture in a T flask or in a plate or dish is based on the amount of space between the cells. The confluency of the culture often influences the growth of the culture and expression.

Anchorage Dependent (Attached) Cells – Cells which require a substratum to divide and produce a monolayer.

Monolayer – A layer of cells one cell thick, grown in a culture.

Suspension Culture – Cells which do not require attachment to substratum to grow, i.e. anchorage independent. Cell culture derived from blood are typically grown in suspension. Cells can grow as single cells or clumps. To subculture the cultures which grow as single cells they can be diluted. However, the cultures containing clumps need to have the clumps dissociated prior to subculturing of the culture.

Density-Dependent Inhibition of Growth – Reduced response of cells upon reaching a threshold density. These Cells recognize the boundaries of neighbor cells upon confluence and respond, depending on growth patterns, by forming a monolayer. Usually these cells transit through the cell cycle at reduce rate (grow slower).

Differentiation – Property of cells to exhibit tissue-specific differentiated properties in culture.

Defreeze (Defroze) – To bring cells out of the freezer; to start a culture from a freezer stockSame as hatch.

Plate – To aliquot cells into microtiter plates; plates can be 6, 12, 96, 384, or 1536 well; as opposed to dishes of either circular or rectangular shape, commonly a 500 cm2 culture dish.

Transient Transfection – The introduction of foreign DNA into a cell to allow the expression of the DNA into the host cell. Protocols are available for opening transient “holes”in the cell membranes allowing plasmids, or siRNA to enter the cell. Cells capable of being transfected or often referred to as “competent cells”. The DNA is not incorporated into the genome therefore, making the event transient referring to the transfection as a transient transfection.

Stable Transfected Cell Line – The selection of a stably transfected cell is where the transiently transfected cells are transfected with a co-expressed selection marker. Typical systems that exist include resistance to antibotics such as neomycin phosphotransferase, conferring resistance to G418, etc. The culturing of the cells can be done as a mixed population or by single cell culture to obtain cell clones from one single integration event.

Indicazioni pratiche

- Camice
- Penna USB

- Turni: vedere foglio excell