Organizzazione del corso

Biotecnologi

mercoledì lezioni 11-13

- 09 marzo
- 16 marzo
- 23 marzo
- 30 marzo

Biologi

mercoledì lezioni 11-13

- 09 marzo
- 16 marzo
- 23 marzo
- 30 marzo

Tutti: venerdì 11 marzo, 09-11, aula C

Gruppo n.1 Laboratorio di colture cellulari 14 - 31 marzo

Gruppo n.2 Laboratorio di colture cellulari 4 - 22 aprile

Programma Lezioni

09 marzo
- le colture cellulari: storia e ambito di applicazione
- basi teorico pratiche delle metodiche relative alle colture cellulari

16 marzo
- l'ambiente adatto per la vita delle cellule
- allestimento di colture cellulari
- criopreservazione

23 marzo
- la sterilità
- Le contaminazioni e la loro identificazione

30 marzo
- metodi per lo studio di proliferazione, adesione, migrazione
- trasfezioni

Programma laboratorio

1° venerdì: presentazione attività
2° venerdì: esercizi di studio di caso

Attività di laboratorio:

- ciascun gruppo di 3 studenti riceve una piastra di cellule in colture:

- mantenimento della linea
- congelamento
- allestimento dell'esperimeto per la curva di crescità
- scongelamento
- marcatura fluorescente dell'actina con falloidina
- conta cellulare

- acquisizione di immagini:
    - a contrasto di fase
    - time-lapse
    - analisi marcatura actina in fluorescenza

Prova di esame

Biotecnologi	Test scritto: - domande su lezioni
Biologi	Test scritto: - domande su lezioni - esercizi di studio di casi - quaderno di laboratorio e report - valutazione pratica individuale

1858: **Virchow** described cell division and showed that all cells arise from other cells.

1882: **Flemming** described cell division in the human cornea and introduced the terms “Mitosis” and described the nucleus structure as “cromatin”.

1883: English physiologist **Sydney Ringer** developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside of the body.

S. Ringer

1885 **Wilhelm Roux** removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days, establishing the principle of tissue culture.

Ross Granville Harrison, working at Johns Hopkins Medical School and then at Yale University, published results of his experiments from 1907–1910, establishing the methodology of tissue culture.

•**Cell culture** is the complex process by which cells are grown under controlled conditions.

•In practice, the term "cell culture" has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi and microbes, including viruses, bacteria and protists. The historical development and methods of cell culture are closely interrelated to those of **tissue culture** and **organ culture**.

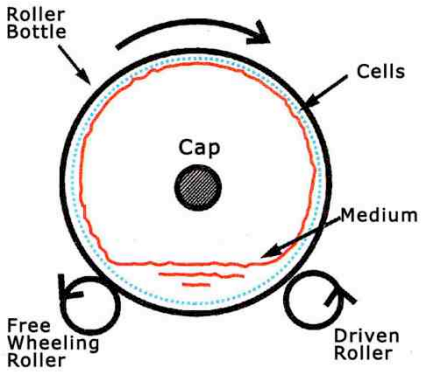
•In modern usage, "tissue culture" generally refers to the growth of eukaryotic cells *in vitro*. It is often used interchangeably with *cell culture*. However, "tissue culture" can also be used to refer to the culturing of tissue pieces, i.e. explant culture or whole organs, i.e. organ culture.It is a tool for the study of animal cell biology in vitro model of cell growth to allow a highly selective environment which is easily manipulated (used to optimize cell signaling pathways).

Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Growing viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The injectable **polio vaccine** developed by Jonas Salk was one of the first products **mass-produced using cell culture techniques**. This vaccine was made possible by the cell culture research of John Franklin Enders, Thomas Huckle Weller, and Frederick Chapman Robbins, who were awarded a **Nobel Prize** for their discovery of a method of growing the virus in monkey kidney cell cultures.

Figure 1. Early cell culture laboratories (circa 1930) at Central Cancer Research Labs which would later become part of the National Cancer Institute. Photos courtesy of NCI Visuals Online; source: G. Terry Sharrer, Ph.D. National Museum of American History, unknown photographer.

Cell culture at research laboratory scale

The culturing of cells can be done on a laboratory scale of an industrial pilot or production scale.



Cell Culture Bioreactors:

A laboratory worker at the German vaccine manufacturer IDT handles a roller bottle to be filled by a robot with sterile cell cultures containing modified vaccinia Ankara (MVA) vector and growth media.

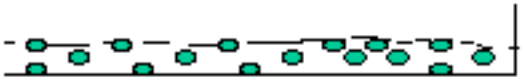
A large Vaccines and Diagnostics Flu Cell Culture Manufacturing Facility

Culture Types

Host

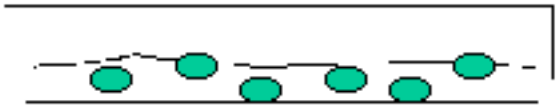
Dissection

Trypsin digestion

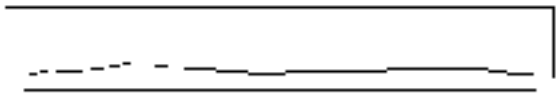


Finely chop

Organ culture



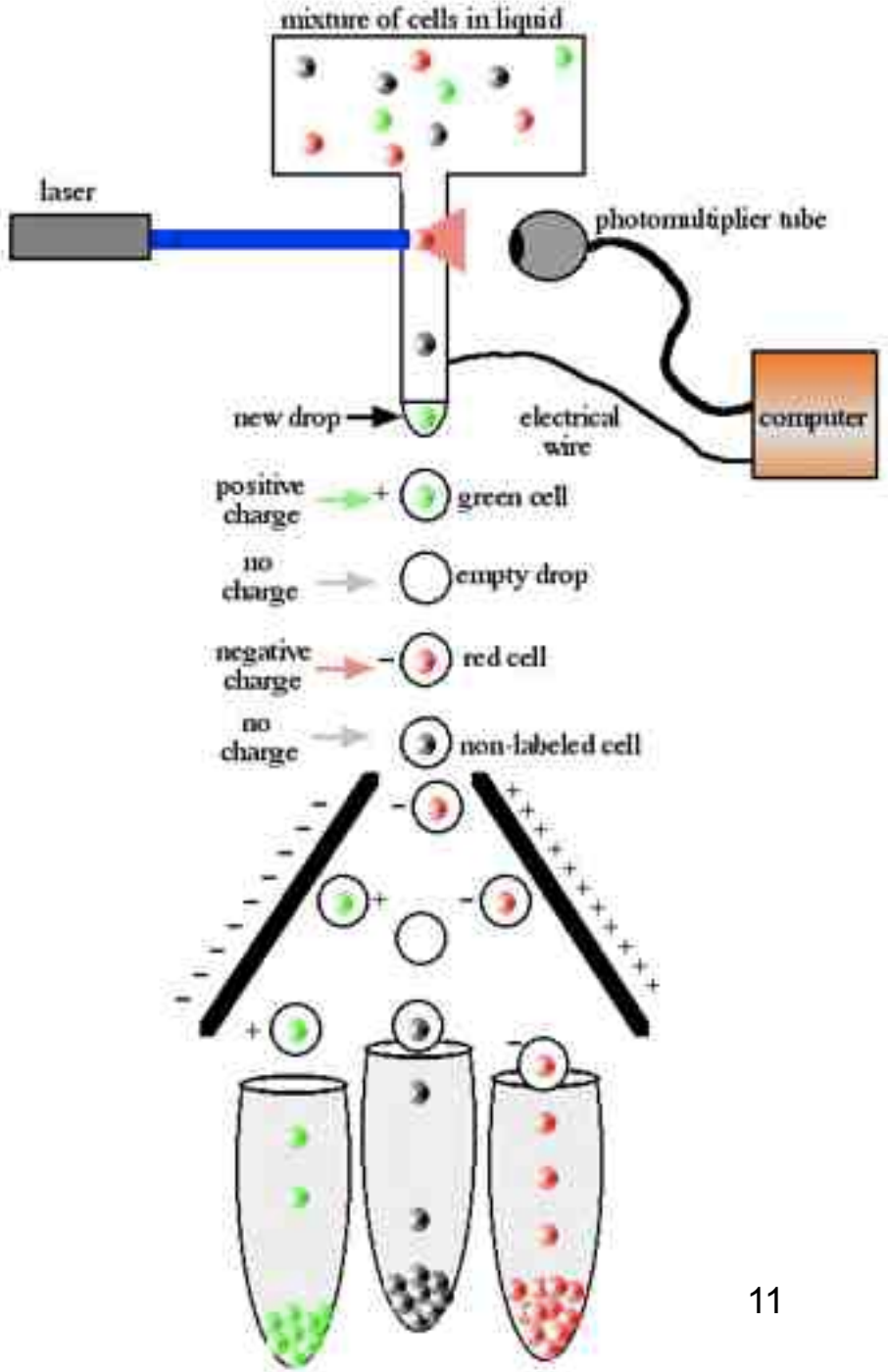
Primary cell culture



Primary explant culture

Separazione delle cellule mediante FACS (cell sorter)

La separazione può avvenire ad esempio grazie all'uso di anticorpi fluorescenti che riconoscono molecole di superficie specifiche della popolazione cellulare d'interesse.

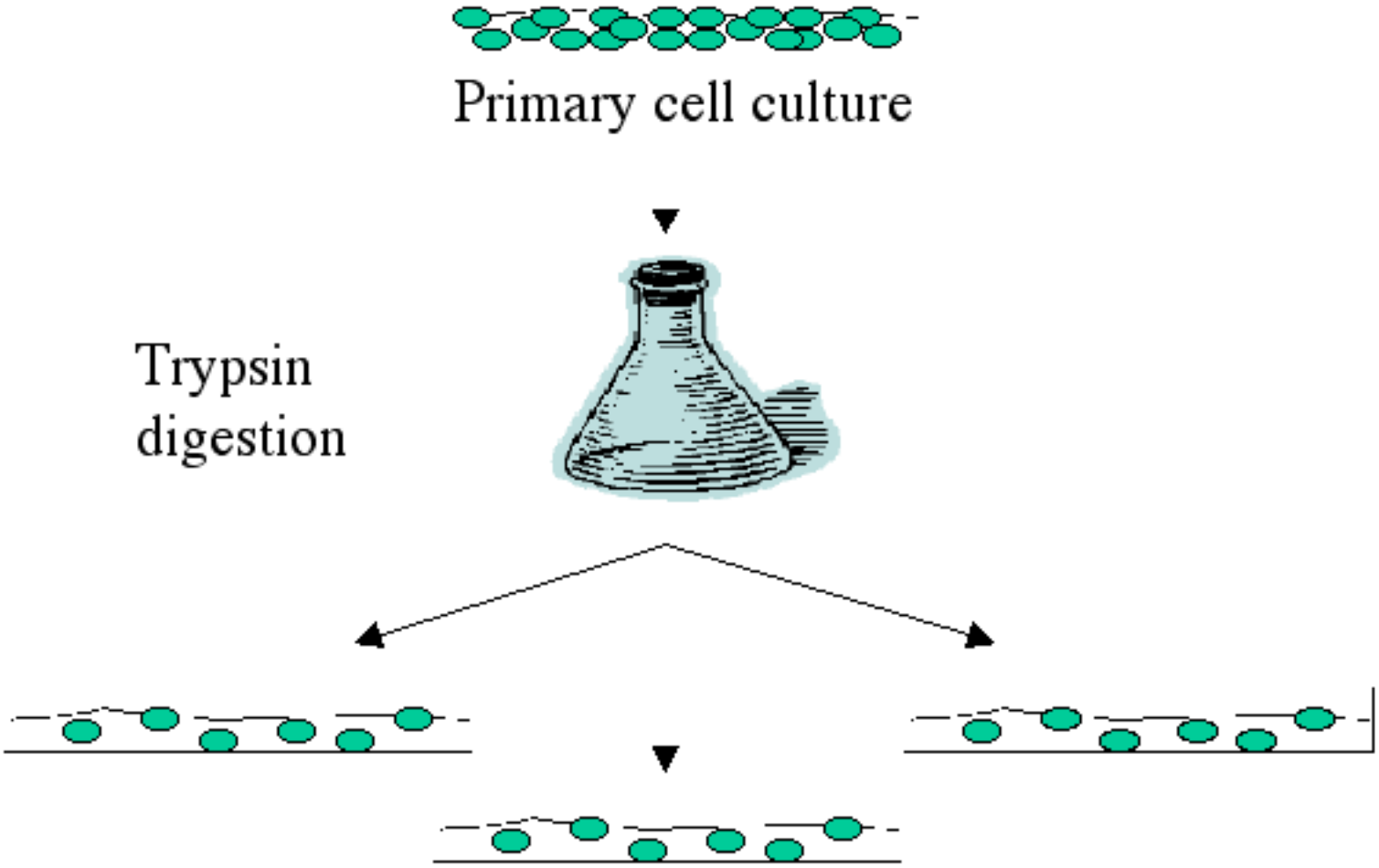


Colture d'organo

mantenimento dei rapporti tra le cellule

nessuna possibilità di **replica**

Cell Lines


Primary cell culture
Trypsin
digestion
Cell line culture
1st passage, 1:3 split ratio

Colture primarie e linee cellulari

possibilità di ripetere gli esperimenti

possibilità di isolare singoli tipi cellulari

perdita di rapporti diretti tra le cellule

le linee sono "finite"

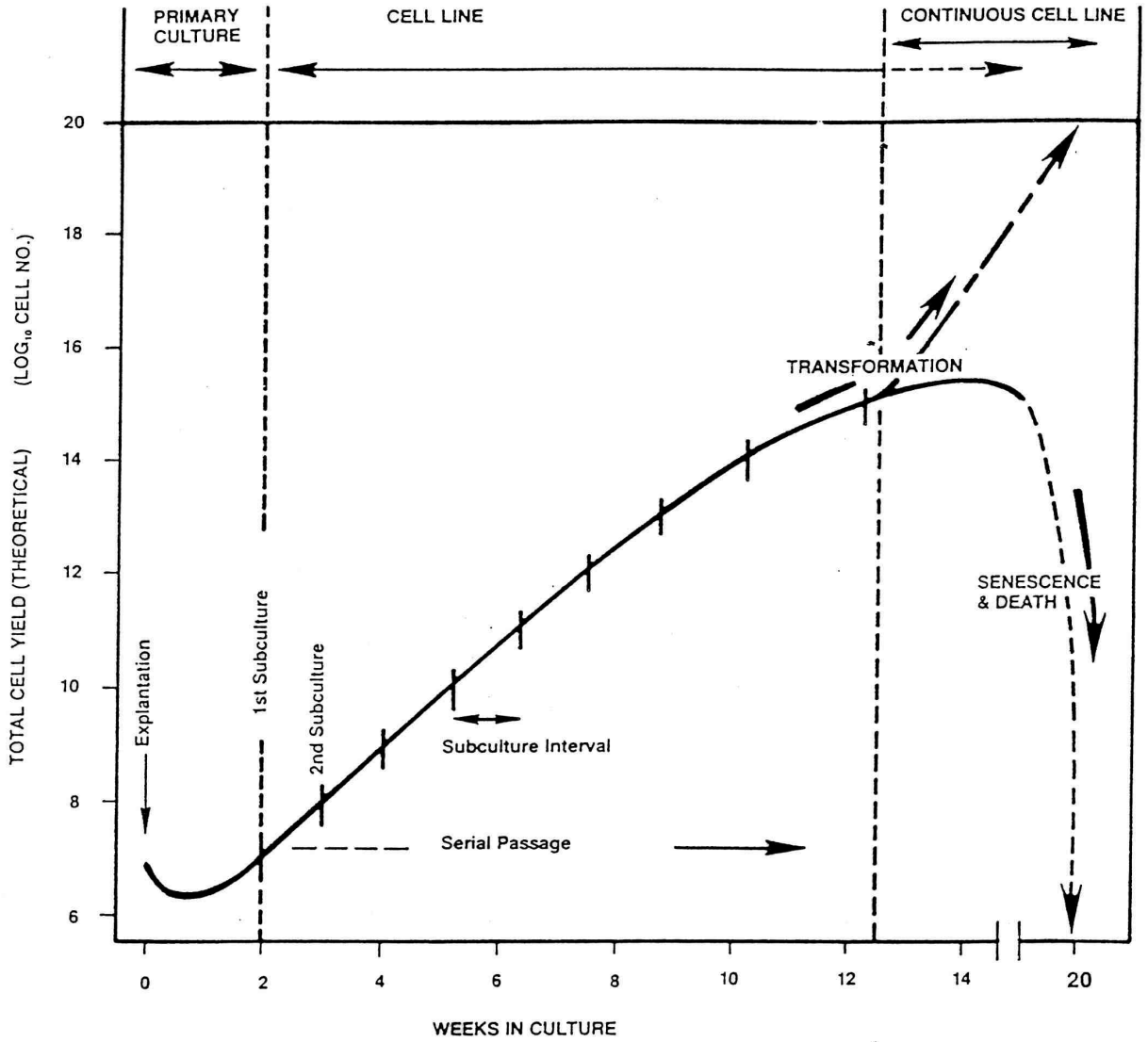


Fig. 2.1. Evolution of a cell line. The vertical axis represents total cell growth (assuming no reduction at passage) for a hypothetical cell culture. Total cell number (cell yield) is represented on the Y-axis on a log scale and time in culture, on the X-axis on a linear scale. Although a continuous 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.

Vantaggi ⟷ svantaggi

Sistemi semplificati rispetto all'organismo in toto
Costituite da unità viventi organizzate
Buon controllo delle condizioni fisico-chimiche e fisiologiche
Caratterizzazione del campione e omogeneità
Versatili: uso in diversi campi
Metodiche relativamente semplici
Riduzione dell'uso di animali e abbassamento costi
Riproducibilità
Linee immortalizzate per definizione non sono cellule "normali"

Non permettono di rispondere ad alcune domande:
- effetti tossici mediati da altri tipi cellulari o tossicità cronica
- correlazioni tra concentrazioni in vitro ed in vivo
- Variabilità delle colture nel tempo