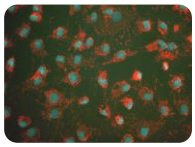
Cell Culture Workshop

Lecture: 2



General Applications of Mammalian Cell Cultures

by

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- **Important applications of mammalian cell cultures include:**

1- Virology and Vaccines Production.

2- Drug Discovery and Screening.

3- Toxicology Testing.

4- Cancer Research.

5- Biotechnology and Tissue Engineering.

6- Genetically Engineered Proteins.

7- Model System.

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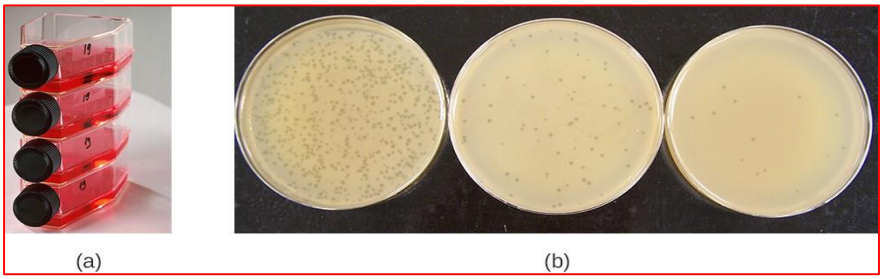
1- Virology and Vaccines Production

• Cell cultures has become a very **useful** and **convenient** method for isolating viruses since **1949** when **Enders** successfully cultured **polio viruses** on neuronal cell culture.



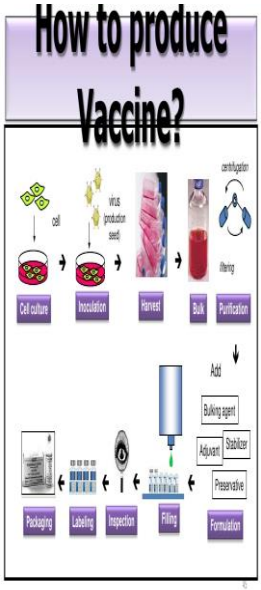
(a) (b)

• **Since that time**, cell culture still remains the **"gold standard"** for isolating many viruses **however** the more modern diagnostic virological techniques such as **PCR**, **EIA** and **IF** as:

i. A **single cell culture** can cultivate **a broad** spectrum of viruses.

ii. Viral cultures facilitate production of **high tittered viruses** used in Abs **testing**, viral **characterization** and molecular **analysis**.

- **Regarding vaccine production**, cell cultures have been used to produce **virus-based vaccines** since early 1950s.
- Vaccine production is **the first industrial application** of animal cell culture technology.
- In which, numerous **vaccines** including **polio**, **measles**, **rubella**, **mumps**, **chicken pox** and **rabies** vaccines have been generated in animal cell cultures.



2- Drug Discovery and Screening

- Cell cultures play an **initial** and **crucial** role in drug candidate **development** and **screening**.
- They are **superior** to ***in vivo*** models as <u>they</u>:

- More **efficient**.

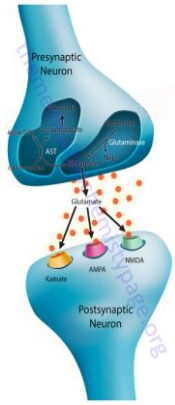
- **Costless** (low compound requirement and short duration).

- Escape **ethical** issues.

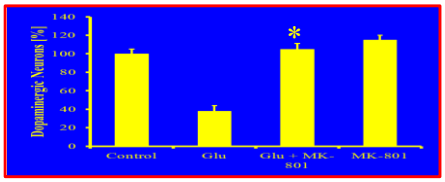
- **For example:** Testing the potential neuroprotective effect of **rotigotine** against glutamate toxicity in primary mesencephalic cell culture (a **PD** *in vitro* model).

- Glutamate is an **excitatory** neurotransmitter released by **glutamatergic** neurons in the brain.

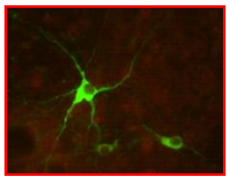
- Upon **elevation**, glutamate can result in degeneration of **DAergic** neurons by stimulation of **NMDA** receptors which in turn increases **OS** leading to **mitochondrial damage**.

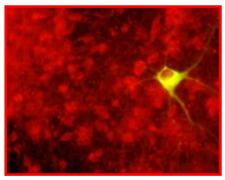


- Treatment of primary mesencephalic cell culture with **0.5 mM glutamate** on the 10th DIV for 9 min followed by a 2-day recovery period resulted in:

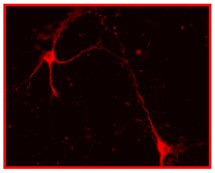


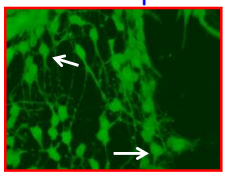
Stimulation of NMDA receptors



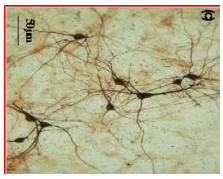


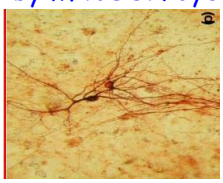
↑ $O2^{-\cdot}$ as measured by MitoSOX dye





↑ Ca^{2+} influx into DAergic neurons as measured by **Fluo-4 dye**

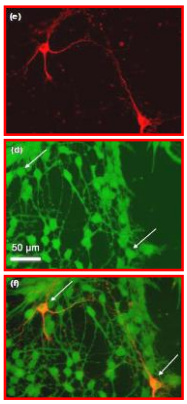




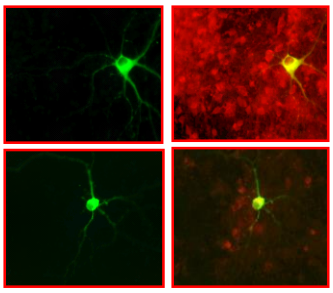
↓ DAergic neurons by 62% compared to untreated controls

1- Disease modeling

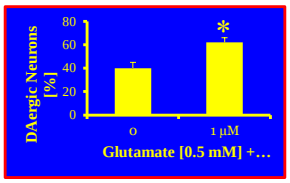
- When cultures treated with **rotigotine** (1 μM) on the 9th DIV and **glutamate** (0.5 mM) on the 10th DIV for 9 min, after two days recovery, **rotigotine caused**:



Significant ↓ in Ca^{2+} influx into DAergic neurons

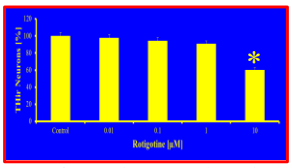


Significant **inhibition** of glutamate-induced O^{2-} production



Significant **protection** of DAergic neurons against glutamate toxicity

2- Target Identification



Rot for 8 days produced no effect on DAergic neurons except at 10 μM

3- Safety Assessment

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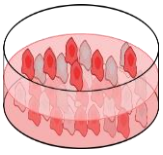
3- Toxicology Testing

- As nowadays **thousands** of compounds are released in **therapeutic** and **industrial** sectors.
- Therefore, there is an **urgent** need for a **reliable**, **efficient**, **fast** and **costless** tool to screen these compounds for their potential toxicity.
- Again, **cell cultures** are considered more **superior** to animal models as they more **efficient**, **time** and **cost-effective** and escape **ethical** issues.



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• **Two types of toxicology tests can be done on cell cultures:**

i- General toxicity tests:

- Carried out on **many cell types** (e.g. fibroblast, HeLa and hepatoma cells).

- **Measuring** viability, cytosolic enzyme release, cell growth etc.

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ii- Organ-specific cytotoxicity tests:

Done on **specialized cells** and measure specific cell functions (e.g. **glycogen** storage in primary hepatocyte cultures, **beating** rate in myocardial cells and **phagocytosis** in macrophages).

4- Cancer Research

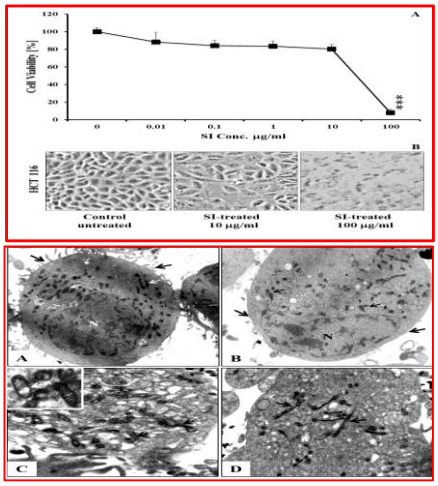
• "Almost **all malignant tumor** entities were established as **immortal** cell lines, and many of them are available **commercially**"…. This help in:

- Studying the **behavior** of different tumors.

- Effectively evaluating **carcinogenic potentials** of different agents.

- Studying the **protective roles** of various natural and synthetic substances against cancer.

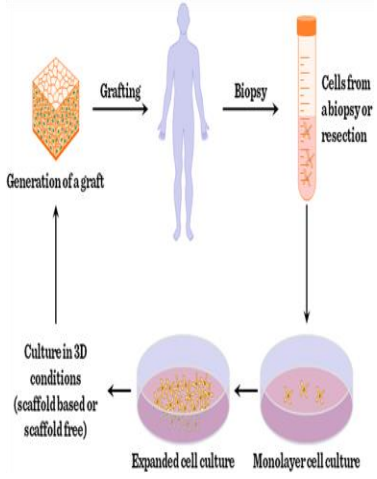
- **For example:** *Solanum incanum* aqueous extract has an ***in vitro*** anticancer activity against colon **HCT 116** cell line by decreasing **cell proliferation** and **induction** of cell death.



S. incanum aqueous extract significantly reduced cell **viability** of cultured cells and caused **loss** of the surface **microvilli** and **mitochondrial** damage

5- Biotechnology and Tissue engineering

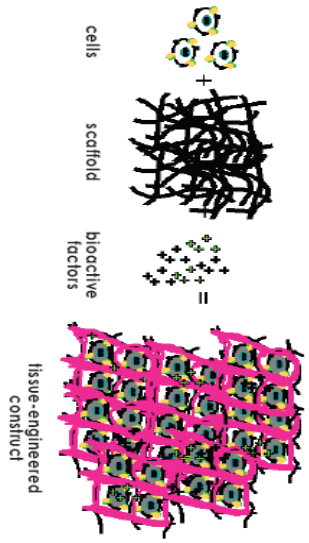
- Simply, tissue engineering means the **re-constitution of human tissues** from the combination of **cell types** grown in culture.
- Tissue engineering is an **important prospect** for future therapeutic treatment with **organ failure**.



• **Two main approaches** are utilized to produce **engineered tissues**:

- **Generating scaffolds** as a cell **support device** upon which cells are seeded *in vitro*. Then, cells are encouraged to **lay down matrix** to produce the foundation of a tissue for transplantation.

- Using the **scaffolds as a growth factor/drug delivery device**. In which, the scaffolds being **combined** with growth factors. **So upon implantation**, body cells **recruited** to the scaffold site and form a tissue.



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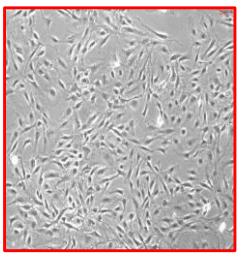
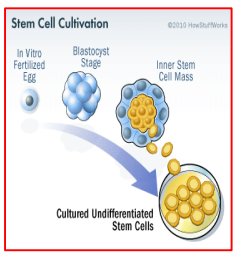
• **Sources** of cells for **tissue engineering** strategies:

- **Primary cells** taken from the patients in conjunction with **scaffolds** to produce tissue for re-implantation (**limited** invasive nature & the potential to be **diseased**).

- **Stem cells** including **embryonic** stem cells, bone **marrow** mesenchymal stem cells and **umbilical** cord-derived mesenchymal stem cells.





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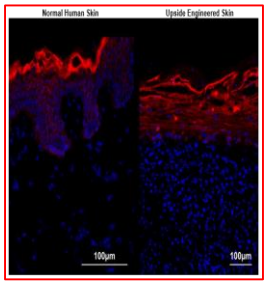
- The more current **successful** approaches:

- Using **primary chondrocytes** for the replacement of damaged cartilage.

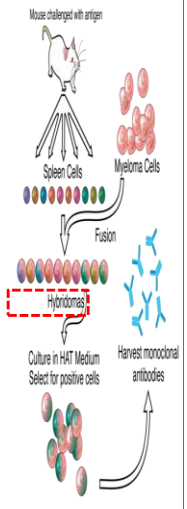


- **Skin cell sheets** for damaged skin.

- **Reconstruction** of some **larger** and **complex** tissues, more notably, the **bladder**.

6- Genetically Engineered Proteins

Cell cultures can be used to produce **proteins** that have **medicinal** or **commercial** values including mABs, insulin, hormones etc.



i- **Production of monoclonal antibodies (mAbs):**

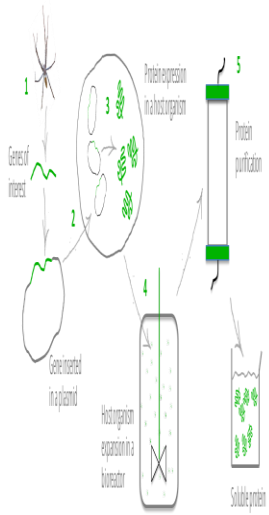
- **mAbs** are produced in large amount by using **hybrid cells**.

- **Hybrid cells** prepared by fusion of **Ab-producing cells** from immunized mouse with a **tumor cells** called **myeloma cells**.

i- Production of recombinant proteins:

- Based on **transferring** of cultured cells with an **isolated gene** and **amplify** it → **expression** of high level of the **corresponding protein**.

- **e.g., interfereone, tissue plasminogen activator** and **clotting factors.**



7- Model System

Cell cultures provide **a good model system** for studying:

1- Basic **biology** and **biochemistry**.

2- The **interaction** between **disease-causing agents** and **cells**.

3- The process and triggers of **aging**.

4- **Nutritional** studies.

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