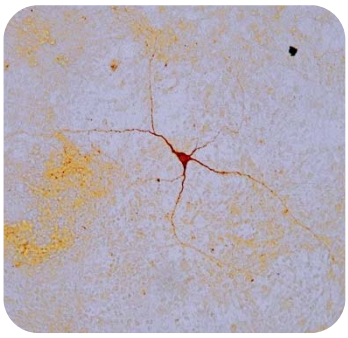

Workshop

Cultivation of Mammalian Cells: Primary Cultures as a Tool in Life Science Research

Nov 26-28, 2017

Workshop part I:

Lectures

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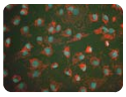
Nov 26-28
2017

Cell Culture Workshop

Lecture:



Cultivation of Mammalian Cells: Primary Cultures as a Tool in Life Science Research

Rudolf Moldzio

vetmeduni vienna

Nov 26 2017

Cell Culture

- ability to maintain cells outside the body in an determined and well characterized environment
- possibility to cultivate and work with cell populations under reproducible and defined conditions

Lab for Sterile Working

- separated room, only for cell culture purposes
- economically equipment (only a few devices)
- closed doors and windows
- laminar flow
- incubator
- sterilizer
- autoclave

General requirements

three defined working areas

- cleaning area
- prearrangement area
- sterile area

General requirements

three defined working areas

- cleaning area
  - lab dish washer
  - dryer
  - autoclave
  - storage of lab ware

General requirements

three defined working areas

- cleaning area
- prearrangement area
  - workbench
  - gas-, water- and electricity supply
  - storage compartment
  - vortex
  - fridge / freezer (4°C / -20°C)
  - deep freezer (-80°C)
  - magnetic stirrer
  - pH meter

General requirements

three defined working areas

- cleaning area
- prearrangement area
- sterile area

- flow
- incubator
- water bath
- centrifuge
- microscope
- storage compartment

Why to use Cell Cultures

- cell culture studies are mandatory for the development of new drugs

- studies for regulatory more and more evolve into an animal replacement direction

Why to use Cell Cultures

- the designs of animal studies are often unacceptable (lack of suitable controls, housing problems, inappropriate parameters measured)

- animal experiments are very expensive due to the abundant mandatory tests (hormon levels, clinical parameters, surgical interventions)

- ethical issues

Why to use Cell Cultures

Before you start, you have to decide, what kind of cell culture you want to use

Types of Cell Cultures

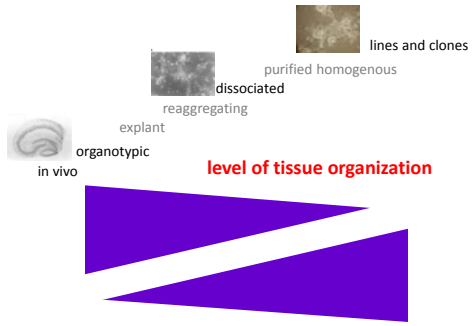
- **Primary Culture**

tissue taken directly from an animal

- **Cell Lines**

immortal/immortalized cells

What kind of culture shall I use?



Pitfalls

- it is well known that cell lines lose some characteristics after passaging more than allowed. So, some parameters studied to confirm the suitability
- primary cell cultures do reflect the in vivo situation properly, but they tend to show accelerated aging
- also primary cells lose some characteristics during cultivation
- In cultures, an increased cell degeneration occurs that influences the outcome of the study

Important rules

- lab safety is mandatory

There are rules about decontamination, resistance against antibiotics, handling of animals and others

Important rules

- GLP „good laboratory practice“
- GCCP „good cell culture practice“
- GSP „good scientific practice“

Three important tools for standardization

Important rules

standardization:

- control of materials (lot numbers etc.)
- cleaniliness
- documentation (data management)
- quality management

Important rules

quality management:

- SOP (standard operation procedure)
- using reference substances
- maintenance of equipment
- good instruction of employees
- use of blinded studies (cross studies)
- statistics

Before you start

you have to decide,

- how long you will incubate with your substance of interest (acute or chronic administration)

Before you start

you have to write a schedule

- the study design includes all groups and all controls

Whatever you do, please use duplicates and all necessary controls

Use positive and negative controls

Randomize your probes

Controls

for colorimetric assays (ELISA etc.)

- Medium control without cells
- Medium control without cells with vehicle

for all tests

- Control with cells without vehicle
- Control with cells with vehicle

Start with pretty simple tests

- **reduced growth rate**
  resazurin, BrdU
- **breakdown of membrane permeability**
  JC-1, PI
- **tissue specificity of response**
  comparison of different cell lines
- **ability to metabolize toxic compounds**
  quantification of compound in cell suspension and supernatant
- **genetic effects/mutagenicity**
  molecular biology

Cell Culture Application