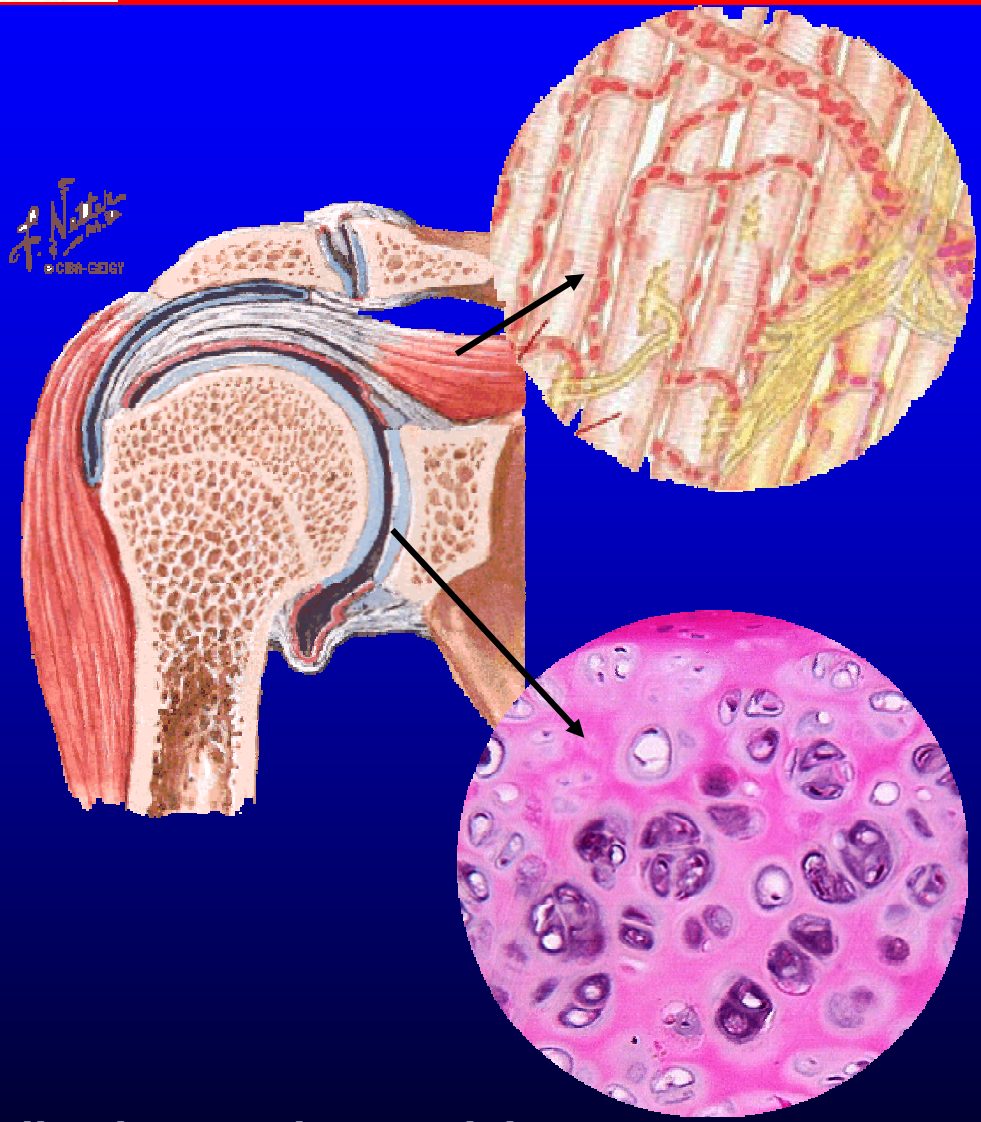
# Methods of Biomaterials Testing
# Lesson 2

Cell Culture and Tissue Engineering

# Tissue Consists of Cells

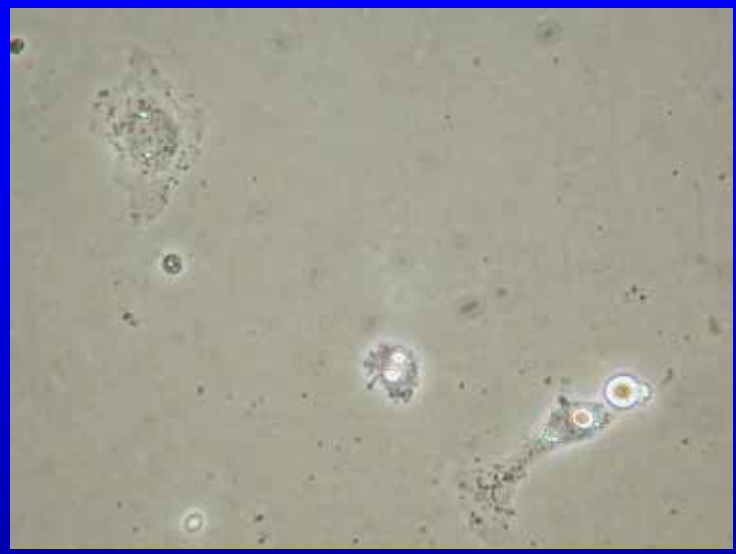


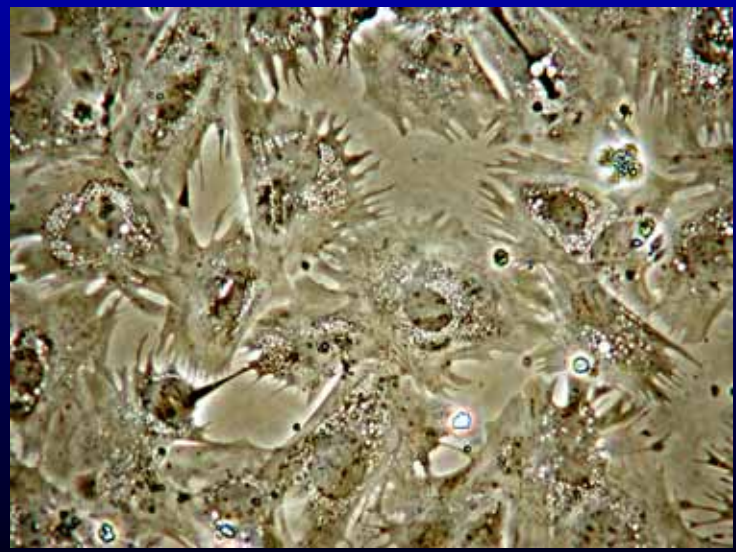
## Tissue consists of

- **Cells**
  - Specific function
    → Parenchym
  - Structure
    → Stroma, mesenchym
    fibroblast/-cyte: tendon, connective tissue
    osteoblast/-cyte: bone
    chondroblast/-cyte: cartilage
    adipocyte: fat tissue
- **Extracellular matrix**
  - water
  - Collagenes
  - Proteogycanes
  - Calcium Phosphates
  - Soluable substances, mediators

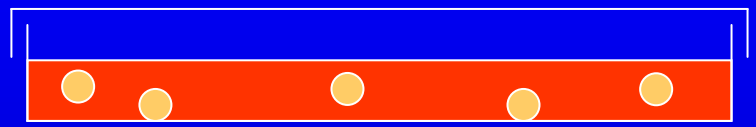
**Cells show main reactivity**
→ Use isolated cells for biomaterials testing

# Bovine Retinal Pericytes

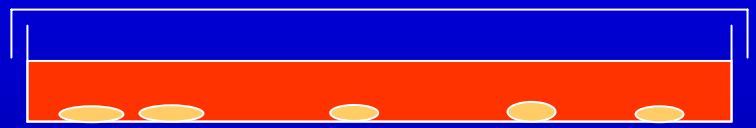




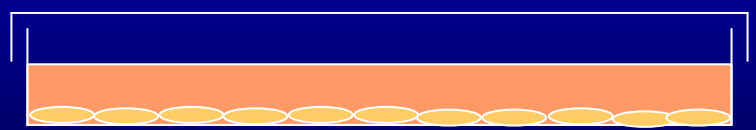
# Cell Culture



$10^5$ to $10^6$ cells/ml in suspension

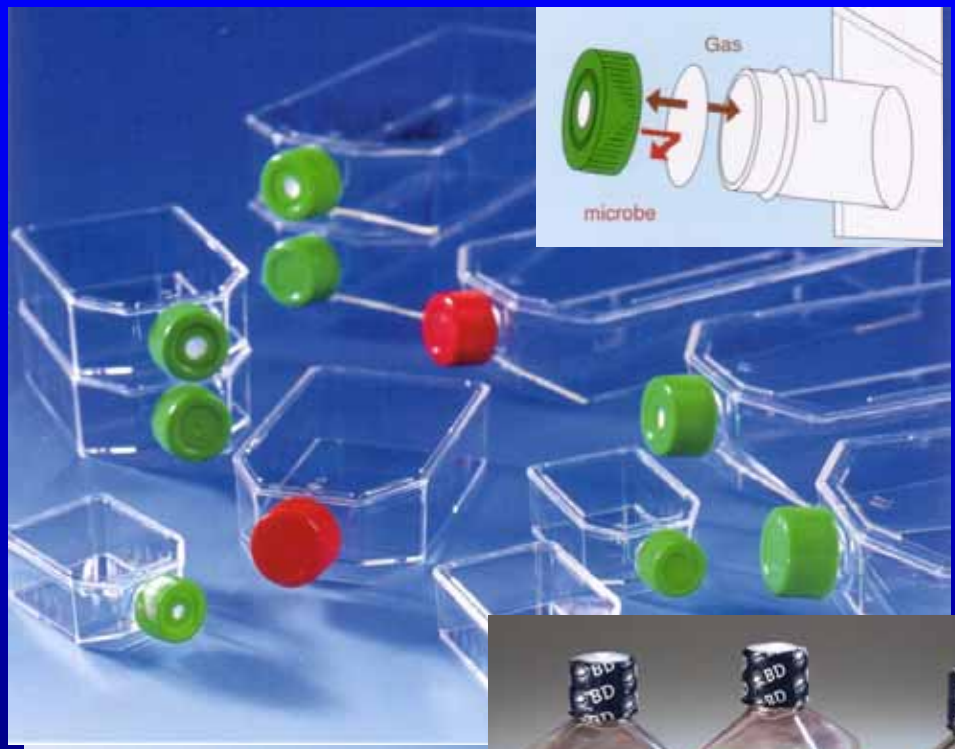


~ $10^6$ cells/$cm^2$ adherent, spread out

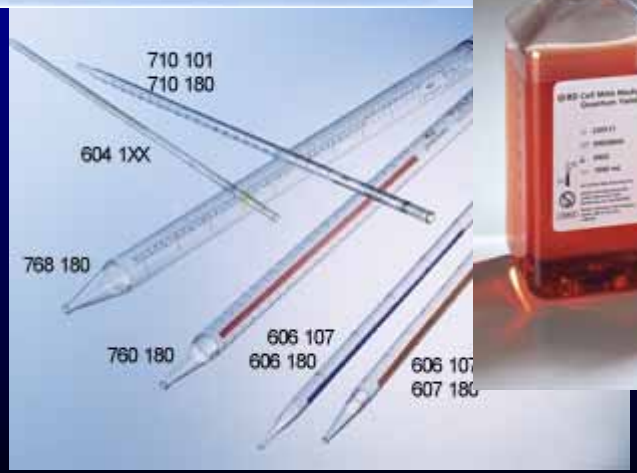


Confluent cell layer

# Cell Culture Equipment



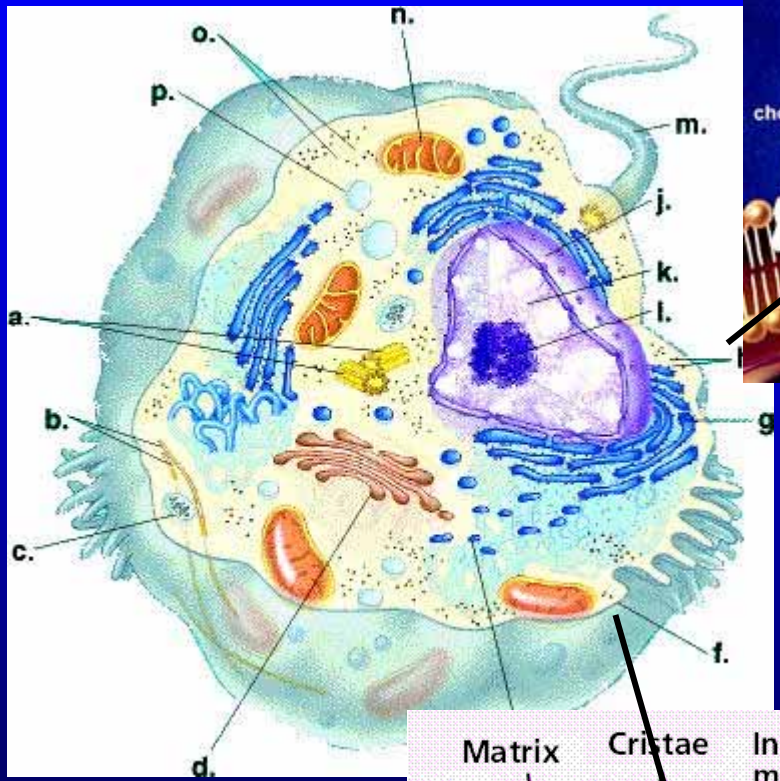


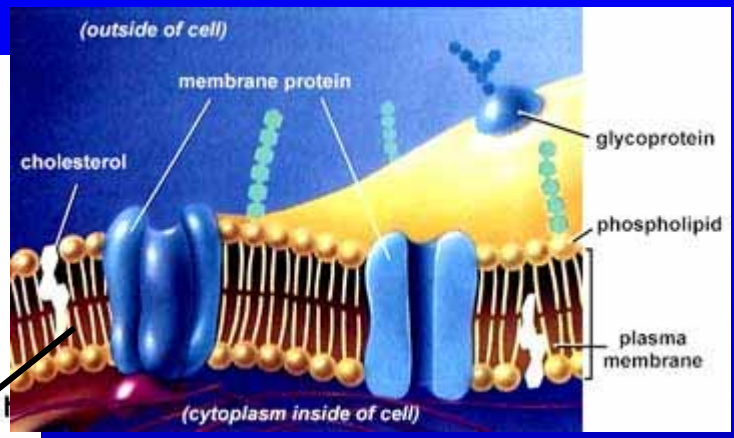

## Cell Culture Medium

- **Nutrients/ Glucose**
- **Vitamines**
- **Amino acids**
- **Salts**
  - Osmotic pressure 300mOsm/L
  - pH buffering salts
    $H_2O + CO_2 \Longleftrightarrow H_2CO_3^- + H^+$
- **Phenol Red**
  - pH indicator
- **Fetal Bovine Serum**
  - **Var. Growth factors**
  - **Var. Proteins**
- **(antibiotics)**

# Organelles of the Cell



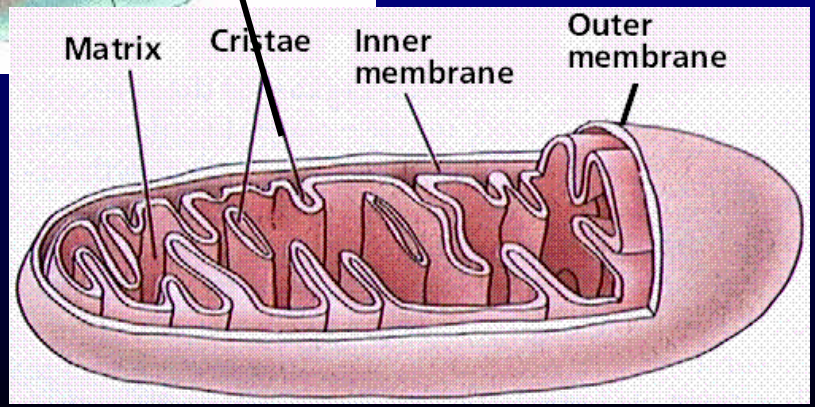

## Cell Membrane



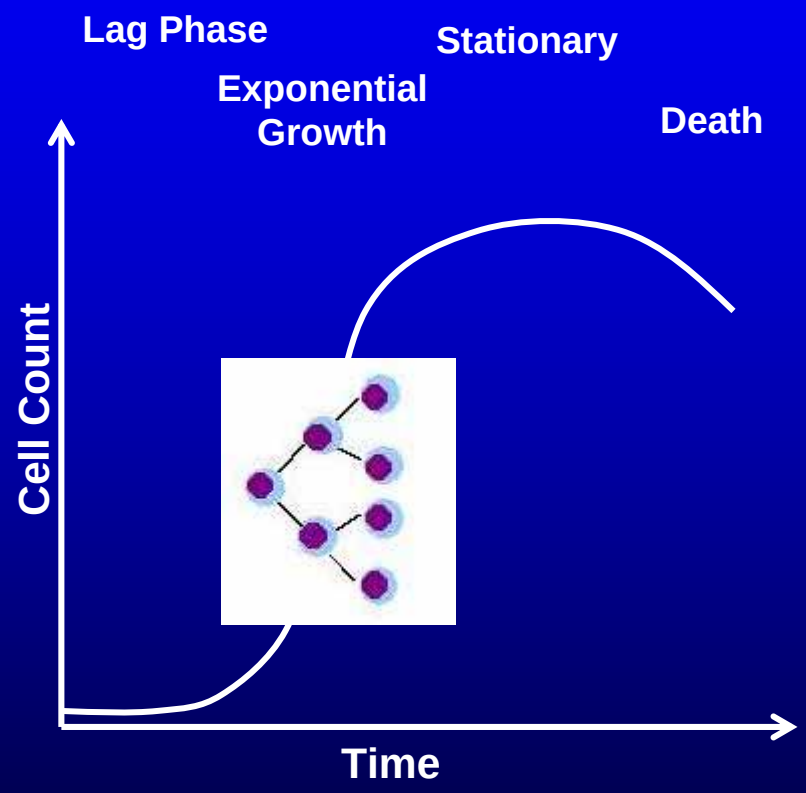

**Semipermeable membrane:**

- **Freely permeable for water**
- **Controlled permeability for ions, messengers via gates, pores**

## Mitochondrion



# Cell Growth

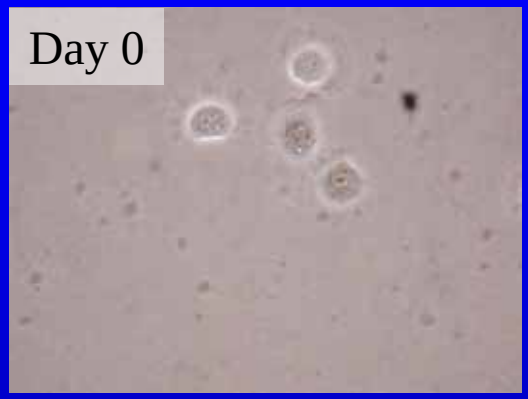


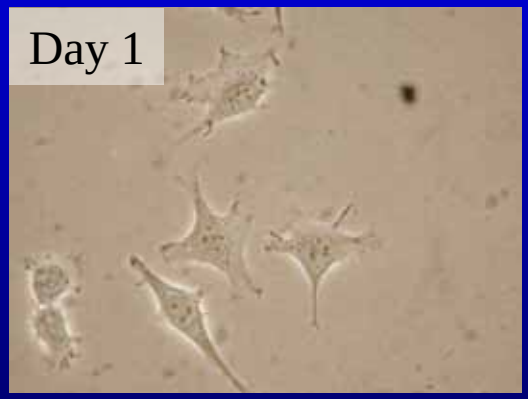

- Lag Phase
  - Low cell growth
  - Cells adapt to the environment
- Exponential growth
  - Maximum cell growth
  - Ideal phase for experiments
- Stationary Phase
  - No more cell growth due to contact inhibition
- Death
  - Death exceeds cell growth

# Types of Cell Culture

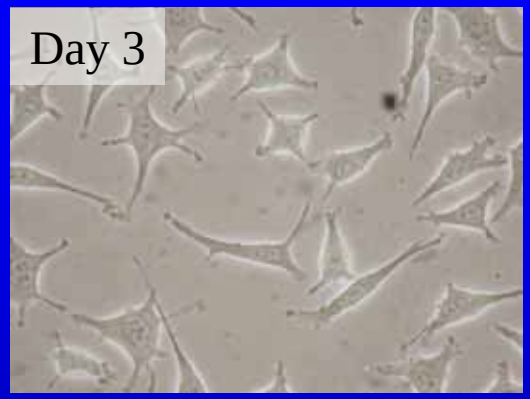
- Culture of Cell Lines
  - commercially available
  - highly standardized
  - immortal
  - as a model more or less far from reality
- Primary Cell Culture
  - Material from individual donors with biological variability
  - Standardized protocols
  - slow growth, limited lifetime
  - closer to reality, interactions between different cells take place
- Tissue Engineering (3 dimensional)
  - little standardization
  - few protocols
  - difficult
  - can be close to reality

# Cell Culture – L929

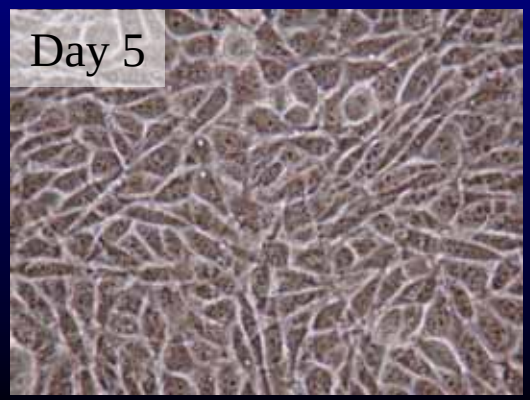


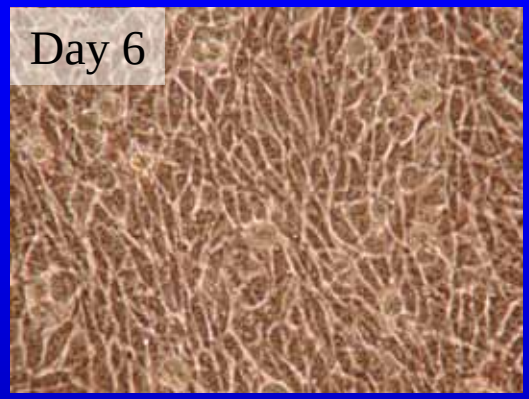



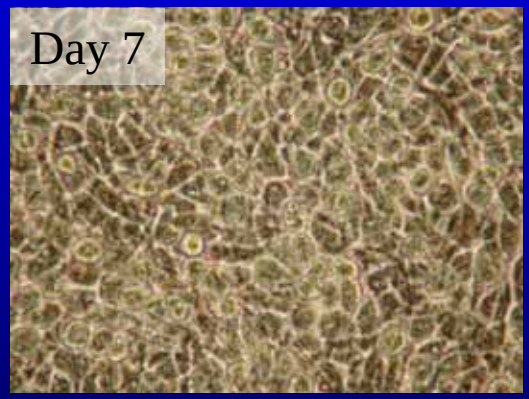




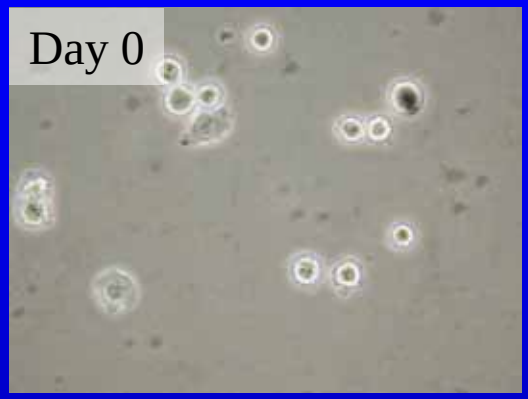




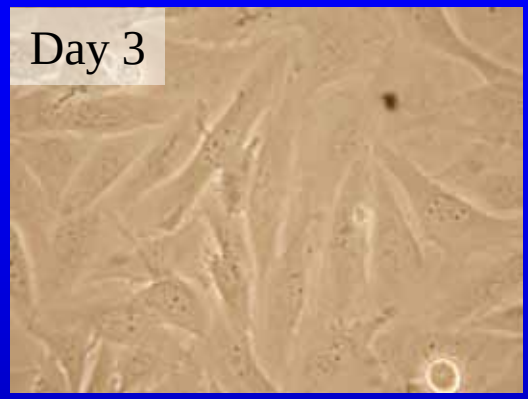



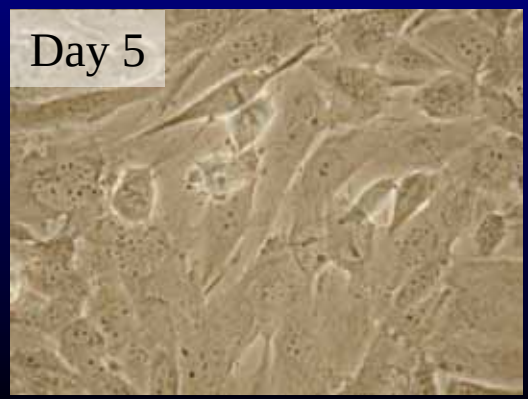
# Cell Culture – SAOS-2

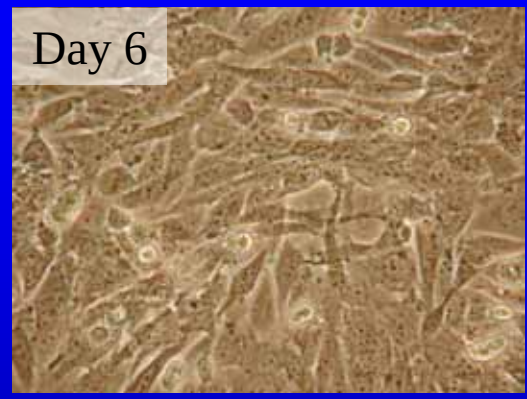


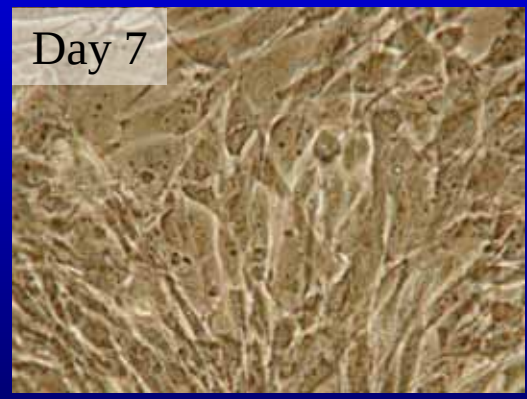


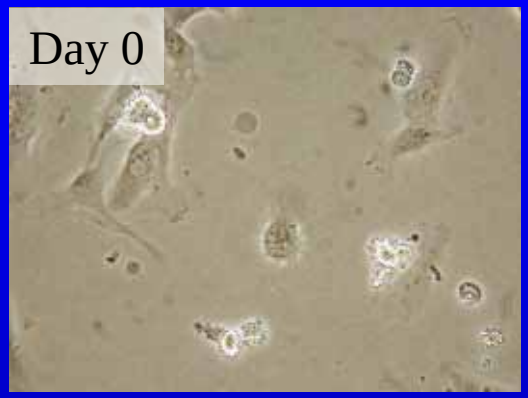




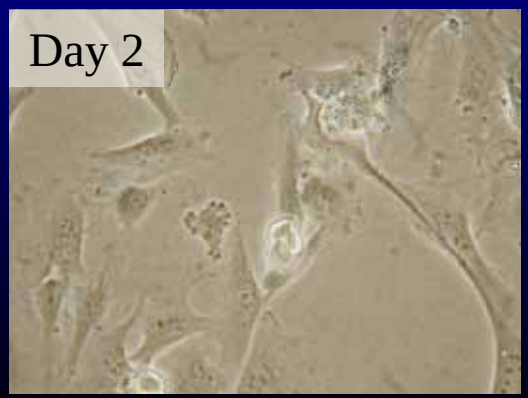



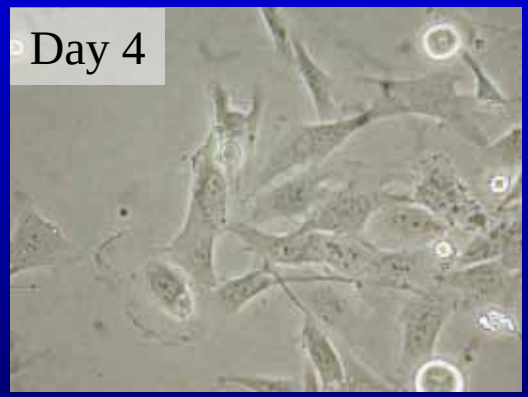
# Cell Culture – HMEC-1



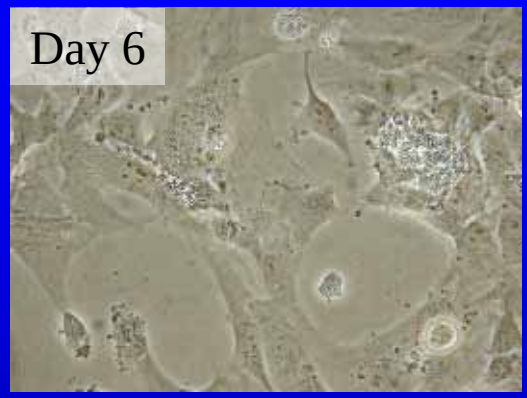













# Sterile Working

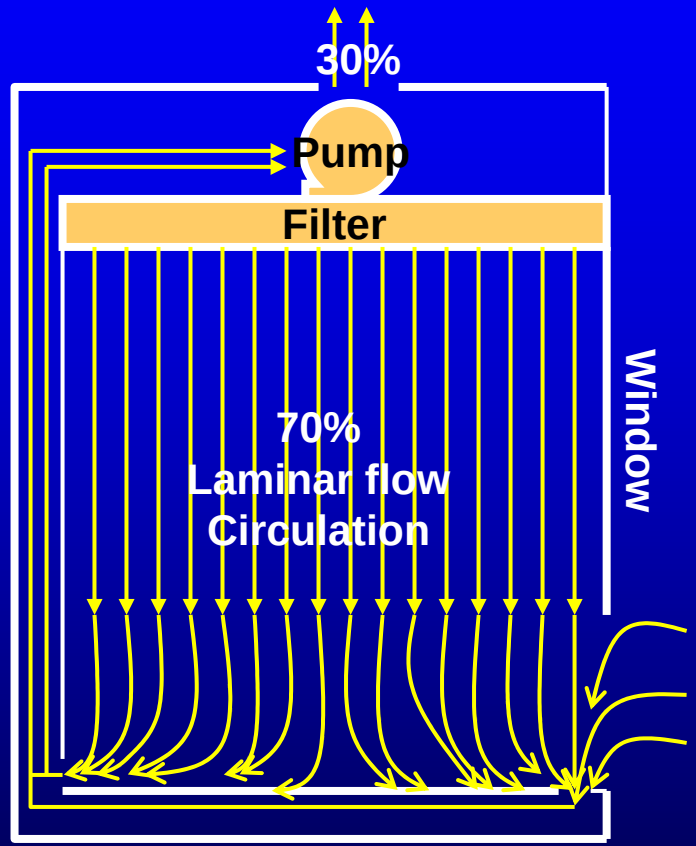
## Purpose

- Cell culture has no immune system or other type of immune protection
- Bacterial doubling time is much faster 20min vs. 1 day
- Bacteria/fungus/yeast directly influence cells
- Bacteria/fungus/yeast release toxic substances

## Method

- Desinfection of the Working place
  - 70% Alcohol
- Work on a Laminar Flow Bench
  - Sterile filtered air
- High discipline
- Sterile, single-use equipment
- Sterilize other equipment
  - Dry heat
    - 180°C, 2 hours
  - Autoclavation
    - 121°C, 2bar $H_2O$ vapour
  - Ethylene oxide, Formaldehyde
  - Irradiation
- Sterilize liquids
  - Autoclavation if heat resistant
  - Filter sterilization
- (antibotics)

# Laminar Flow Bench (S2)



# Autoclave

# Disadvantages of standard Cell Culture

- Only one cell type
  - No communication/ interaction of cells
  - Not the original composition of the extracellular matrix

  →Co-culture

  →Animal experiment
- Only two-dimensional cell growth

  →Tissue Engineering: seeding cells on a 3D scaffold
- Static conditions
  - No load, no shear forces
  - Dynamic cell cultures, culture in flow chambers...

# Cell Co-Culture I

Grow several cell types together in one medium

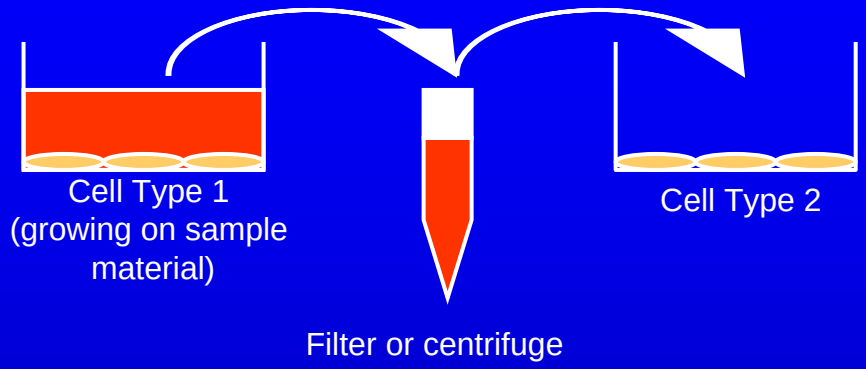
**Purpose**: Check the influence of one cell-types reaction on an other cell type or use the environment of one cell type, which is necessary for the other

**Problems**:

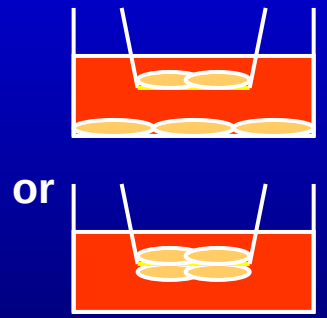
- Because of the different speed of growth of different cells, one soon will overgrow the other
- Cells cannot be identified any more

# Cell Co-Culture II

- Transfer of supernatant
  - Convenient
  - Reliable
  - One-way communication only

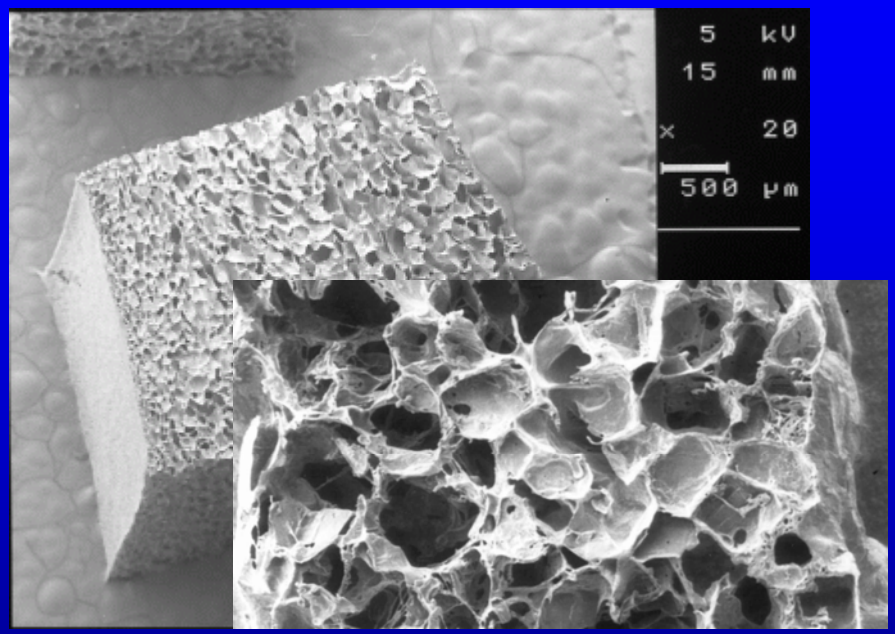


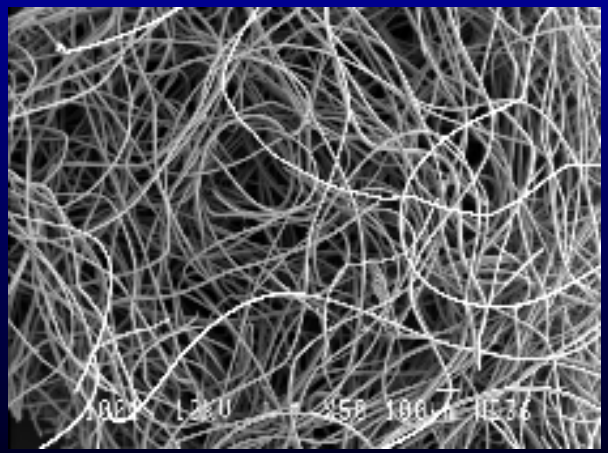

- Co-Culture with membranes, insets
  - Direct contact possible
  - Bilateral communication
  - Expensive
  - Low number of cells
  - Problems with oxygen supply
  - Compared to natural conditions still not the original Extracellular matrix




- Co-culture with Gamma-irradiated cells
  - Mainly for nursing/ feeder cells, which provide environment for other cells

# Tissue Engineering



## Principle

- Seed cells on a porous 3D scaffold

## Technical Problems

- Limited penetration of the cells
- Limited perfusion of the scaffold
  - Oxygen
  - Nutrients
- Perfusion becomes worse with cell growth

## Ways to overcome Problems

- Perfusion chambers
- Seeding endothelial cells first