

Intracellular Transport

- chemical energy (ATP) to mechanical work
- directed motion
- Kinesin: carry out microtubule-based retrograde transport (towards cell edge)
- Dynein: carry out retrograde transport (towards cell nucleus)

Growth and Differentiation

Cell Cycle

G1: Cell growth
S: DNA Synthesis
G2: Growth and preparation for Mitosis
M: Mitosis (cell division)

Quiescent cells: cells pause before replication. Reversible growth arrest (G0 phase)

Transfection

Insert DNA that codes for the wanted biomolecule into cell. Use:

- viruses
- electroporation
- carriers

store transfected cells in cryogenic conditions

Actin Filaments

- Provide support, change the cell shape (division) and drive movement
- Assemble from globular proteins ("G-Actin") like microtubules and form hierarchical structures by crosslinking
- Polar with no preferred direction
- Can form protrusions → exploring and sensing environment
- "Myosin motors" → participate in cargo transport and muscle contraction

Cell Sensing and Signaling

Cellular Communication

Long Range:
 Endocrine • into blood stream
 Neural • in neurons (electric)
 • affect whole organism

Short Range:
 Paracrine • affect local tissue
 Contact Dependent • direct binding

Signalizing

Cell Surface Receptors:

Extracellular signal molecule (hydrophilic or charged) causes receptor to release intracellular signal molecule.

Intracellular Receptors:

Signal molecule (hydrophobic) cross the membrane and act inside the cell.

Signal is:

- Amplified
- Distributed
- Integrated
- Modulated (feedback loop)

Receptors

Ion-channel-coupled

Enzyme-coupled

G-protein-coupled receptors

- Ligands (ex.: hormones, neurotransmitters) bind to GPCR (G-protein coupled receptor) which changes conformation
- Activated receptor causes G-protein to exchange its GDP for GTP gets activated
- G-protein modulates the activity of effector molecules generate intracellular second messenger

Stem Cells – Cell Differentiation

Differentiated cells in adult organisms contain all the genetic information to form a new organism. But, they express **only a fraction** of genes specific to their function.

Stem cells can differentiate to tissue-specific cell, but also renew themselves.

Pluripotent stem cells can give rise to all cell types.
Multipotent stem cells can give rise to a limited number of cell types tissue specific.

Bioprocesses

Bioprocesses rely on several key components, including biological components, such as the target molecule and the cells used to produce it; culture medium and one or more bioreactors; as well as a process.

Modern technology used:

- DNA sequencing and synthesis
- Precise gene editing
- Genetic circuit design

Cell source:

Mammalian cells:	E. coli:
- slow growth	+ fast
- complex growth media	+ simple growth media
+ proper folding	- refolding required
+ glycosylation	- no glycosylation

Culture medium

Source of nutrients to support the growth of cells.
 Composition:

- building blocks (sugars, aa)
- water
- salts/ions

Bioreactor

Carefully designed **culture medium** that provides **nutrients** (building blocks, water, ions, energy) and a **suitable environment** for cells to grow and generate biomolecules of interest.

Batch: Feed solution enters vessel containing cells. No addition.

Perfusion: Fresh medium enters vessel containing cells. Removal of product-rich culture broth.

Cell and tissue architecture

Cytoskeleton – "Bones and muscles of the cell"

- Resistance to deformation
- Drives movement
- Organizes the cell interior (shape and cargo)
- Physical interactions with the environment
- Present in all eukaryotic cells

Intermediate Filaments

- Toughest and most durable filaments
- Primary function: **Withstanding mechanical stress**
- Assembled from α -helical proteins → Rope-like structures
- Without preferred direction (Diameter 10nm)
- Major types: Keratins, Vimentins, Neurofilaments, Lamins

Microtubules

- Essential for spatial organization
- Polar, have a distinct orientation, centrosome → cell membrane
- Are assembled from globular proteins: α - and β -tubulins that assemble in tubulin dimers (25nm diameter)
- Dynamic → constantly grow or shrink
- Can form Cilia, help in cell division or transport

Actin Filaments

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Muscle contraction:

In a **sarcomere**, myosin II binds to actin and proceeds towards the **plus** side. This sliding of myosin on actin filaments **shortens** the strands and leads to muscle contraction

Extracellular Matrix (ECM)

Fibrous elements outside the cell that hold cells and tissues together

Functions:

- Structural support and mechanical scaffold
- Resistance to stretch and compression
- Boundary between tissues
- Water retention
- Reservoir for signaling molecules

Plants have cell walls (cellulose and pectin) instead of ECM.

Composition of ECM

Protein fibres:

Collagen and **Elastin** provide **strength and elasticity**

single collagen polypeptide chain
 triple-stranded collagen molecule
 collagen fibril

Glycoproteins:

Fibronectin and **Laminin** provide **adhesion and signaling**

extracellular matrix binding site (e.g., via collagen)
 cell attachment site (e.g., via integrin)

Glycosaminoglycans (GAGs) and Proteoglycans:

- Linear, rigid polysaccharide chains → form large volumes of porous gels
- They carry **negative charges** → **retention of water**
- Often covalently linked to protein cores called **proteoglycans** that also provides lubrication
- Resistance to compression**

All components are **produced** and **matured** in the cells then **secreted** in extracellular environment
 → Cells engineer their local extracellular matrix

Physical Cell-Cell and Cell-ECM Interactions

Mechanical, electrical, metabolic coupling at cell-cell junctions (desmosome). Physical cell-cell and cell-ECM communication (e.g. via integrins)

Woundhealing and Tissue Engineering

Circulatory system

The circulatory system consists of cardiovascular system and the lymphatic system.

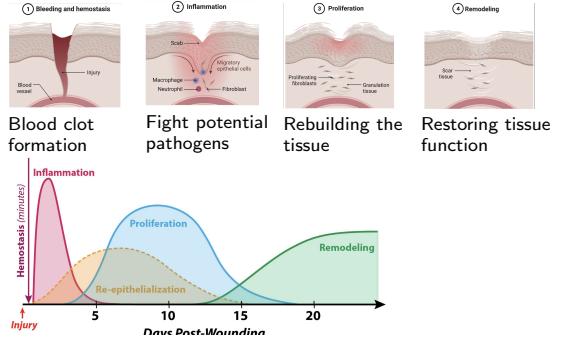
Blood cell types:

- Enucleated: **Erythrocytes** (red blood cells) and platelets
- Nucleated: **Leukocytes** (white blood cells) and other immune cells

Vascular Structure:

Artery, Arteriole, Capillary bed, Venule, Vein

Woundhealing



Tissue Engineering

Mimic in vivo conditions so that cells can grow.

- scaffolds
- genetic tools
- biomaterials
- bioreactors

Hydrogel as ECM mimics:

- Hydrophilic networks with tunable mechanical, biochemical and physiochemical properties.
- Matrices can be natural, engineered or hybrid.
- Body tissues have different matrix properties.

Microphysiological Systems and Immune Engineering

Organoids

Organoids are "mini organs" built in the lab from stem cells.

Organoids **mimic geometric features** and **cell organization** of the original organ (tissue replica). The surrounding **ECM composition** is **custom** designed.

Matrigel: matrix scaffold for organoids, provides **mechanical support** and **adhesion sites** for cells.

Cell source: induced pluripotent SC (iPSC) terminally differentiated cells reprogrammed back to pluripotency.

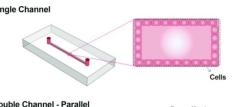


Possibility to model **genetic diseases** such as Parkinson in cerebral organoids.

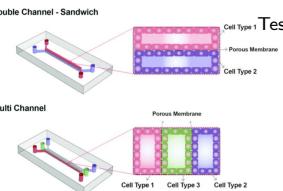
Limited to small size, nutrient and oxygen supply is limited by diffusion.

Organs on a Chip

Micro-tissues grown in a controlled microfluidic device where **physical** and **mechanical stimulation** can be applied. OoAC are patient-specific (gender, age, history of disease, etc).



- Controllable flow circuits
- Multiple tissue compartments
- Multiple cell types
- On-demand drug release
- Small scale but high-throughput



- interaction between different cell types
- safety in wholeisitic concept
- Measure uptake and clearance rates

Immune Engineering

Consists of using engineering tools and principles to investigate and modulate the **immune system**.

Applications:

- Evade or delay immune response
- Shut off auto-immunity in auto-immune diseases
- Stimulate immune response (e.g. vaccines)
- Multiply native immune response (T cell activation)

Immune Response

Self:(part of organism)

- Own organs, cells and proteins
- Commensal bacteria

Non-self (not part of organism):

- Non-harmful particles of food or pollen
- Pathogens (bacteria, viruses...)

Innate:

Unspecific and immediate (hours)

Adaptive:

Specific and slow (days)
Lymphocytes (B and T cells)

Evading (block reaction):

- Avoid cell attachments or phagocytosis (for example through "self"-markers) – physical
- Avoid protein adsorption with hydrophilic, non-fouling coatings – biochemical

Activate:

- Delivery of **cytokines** → stimulate immune cell proliferation and recruitment
- **Vaccines** that expose the immune system to specific antigens
- **Hydrogels** loaded with immune-stimulatory substances

Immunotherapies

- use engineered **antibodies** to boost the immune response
- antibodies can be **modified** and mass-produced to trigger an amplified and **targeted therapeutic response**
- CAR-T cells: New form of cancer therapy where T cells from patient's blood are **genetically modified** to attack specific proteins on **cancer cells**

Foodprocessing



- Yougurt
- Cheese
- Beer
- Milk

Meat alternatives

Plant based meat alternatives, from **pea protein**.

Lab grown meat alternatives, cell cultures grow into synthetic muscle tissue.

Microbiome

All the bacteria and microbes within the GI tract.

Functions:

- Barrier integrity
- Mucus production
- Food metabolism
- Transform food products into chemicals that act as **signaling molecules**.

Dysregulation:

- Leads to diseases (ex: anxiety, depression, insulin resistance, etc)

Solutions:

- **Probiotics**: bacteria that metabolize food sources into signals that regulate homeostasis in body.
- Design **microphysiological models** (eg. OoAC) that capture the interactions between the gut microbiome intestinal cells.

Drug Delivery

Diagnostics

RT-PCR tests: real time polymerase chain reaction

- Amplification of nucleic acids is done through cycles of **DNA elongation** using **DNA polymerase** (temperature controlled)
- Steps: DNA denaturation, primer annealing, elongation of new DNA strand
- At each cycle, the number of DNA fragments doubles (measured optically with fluorescent dye)
- Higher sensitivity than lateral flow tests

Lateral flow tests: rapid antigen tests

- **Detection of proteins** (antigens) through immobilization of a receptor-nanoparticle complex on a substrate.
- **Antibodies bind to antigens** in the sample and **antigens simultaneously bind to capture antibodies** immobilized on test line.
- The **antigen** is sandwiched between the **capture antibody** and the **detection complex**.
- Signal depends on: the amount of virus, flow, diffusion of antigen proteins and kinetics of reception-antigen binding.



Prophylactics

Vaccine breakthrough (COV 19):

Requirements:

mRNA sequences:

- increased stability
- longer half-life
- higher translation efficiency

mRNA sequence carrier:

- Protect cargo
- Carry it across the tissue barrier
- Target specific cells
- Allow the mRNA to escape the endosome

Which **barriers** need to be crossed?

- Extracellular barriers (blood vessels)
- Intracellular barriers (LNP endocytosis)
- Endosome and LNP degradation
- mRNA free in cytoplasm

Lipid Nanoparticles (LNPs)

Needed to **avoid digestion of mRNA** by nucleases (foreign body response).

LNP include 4 main components:

- **phospholipids**: stabilize shape
- **ionizable lipids**: help RNA to escape endosome
- **cholesterol**: reduce permeability
- **PEGylated lipids**: help avoid immune response

How are LNPs assembled?

- **Lipids and cholesterol** are dissolved in an **organic phase** (e.g. ethanol)
- **mRNA** is dissolved in an **aqueous buffer** (low pH)
- **Rapid mixing** of both phases leads to LNPs assembly

Keine Gewähr für Richtigkeit und Vollständigkeit

Viel Spass beim Lernen :))

Neuste Version:

[https://github.com/Skinny-King/Bioengineering/tree/
main](https://github.com/Skinny-King/Bioengineering/tree/main)