

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)
 Proliferation: population \rightarrow

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 \rightarrow exploring and sensing environment
- "Myosin motors" \rightarrow participate in cargo transport and muscle contraction

Cell Sensing and Signaling

Cellular Communication

Long Range:

Endocrine	Neural
• into blood stream	• in neurons (electric)
• affect whole organism	• at synapses (chemical)

Short Range:

Paracrine	Contact Dependent
• affect local tissue	• direct binding

Signal is:

- Amplified
- Integrated
- Distributed
- 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

Growth

Exponential Growth: $N(t) = N_0 e^{rt}$

Logistic Growth: Carrying capacity K , $N(t) = \frac{N_0 e^{rt}}{1 + \frac{K}{N_0} (e^{rt} - 1)}$

Clonal Population: $N(t) = 2^{t/\tau_d} N_0$, Doubling time: τ_d

Clonal Population

Genotype: ensemble of all the genes of a cell ("all available genes")
Phenotype: output of set of expressed genes ("all visible genes")
Clonal population: same genotype and phenotype, identical cells, can differ due to mutations in genotype

Cell Death

Apoptosis:

- controlled cell death
- directed by extracellular signals
- controlled by intracellular signal cascade
- apoptotic cell gets phagocytosed by macrophages

Necrosis:

- death as result of injury
- cells burst and release their contents

Cell and tissue architecture

Cytoskeleton – "Bones and muscles of the cell"

Intermediate Filaments

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

Microtubules

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

Transfection

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: no additions

Fed-batch: small volume of concentrated nutrient solution added

Perfusion: fresh medium addition balances removal of product-rich culture broth

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

Glycoproteins:

Fibronectin and Laminin provide adhesion and signaling

Glycosaminoglycans (GAGs) and Proteoglycans:

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

Physical Cell-Cell and Cell-ECM Interactions

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

Woundhealing and Tissue Engineering

Circulatory system

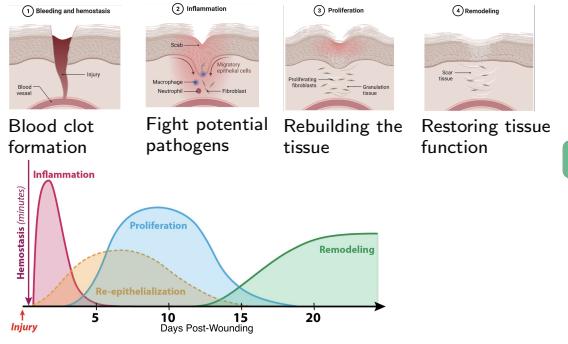
The circulatory system consists of cardiovascular system and the lymphatic system.

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.

- **Biology:** including cells and growth factors
- **Material / Scaffold:** including hydrogels with tunable mechanical properties
- **Engineering tools:** including bioreactors, microfabrication, bioprinting, and perfusion systems

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.

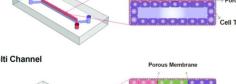
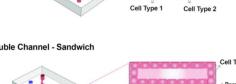
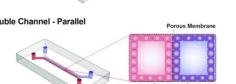
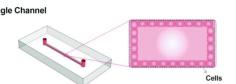


Possibility to model genetic diseases such as Parkinson in cerebral organoids.

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

Testing:

- 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

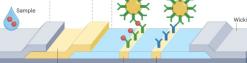
Diagnostics

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) and to facilitate endocytosis.

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)