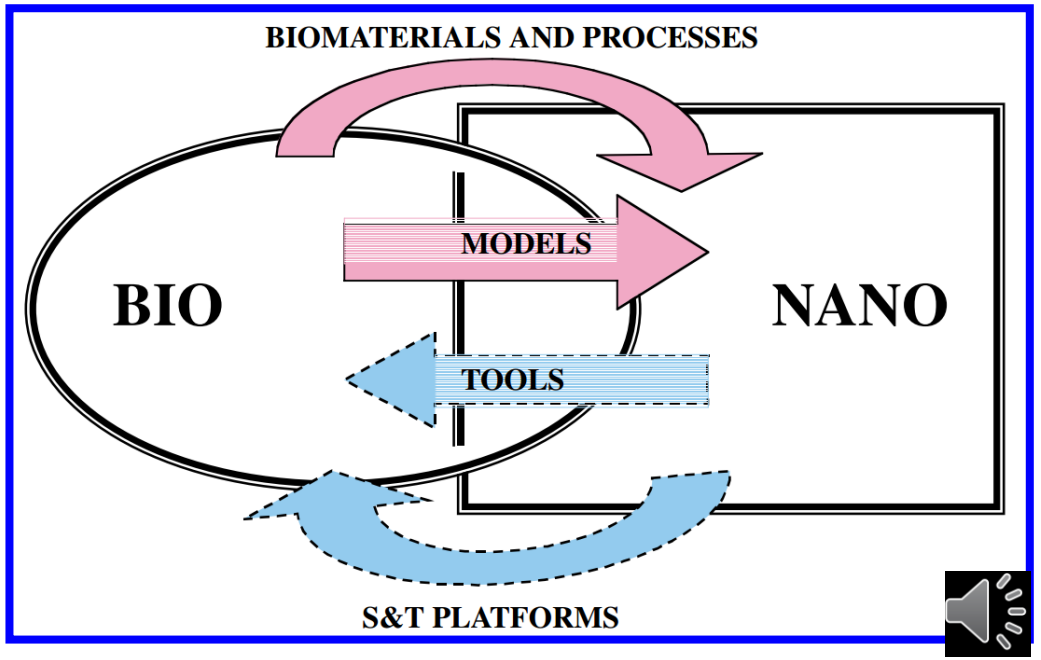
Nanotechnology and Biomedicine

- better understanding and treatment of living systems
- synthesis of new drugs and their targeted delivery
- regenerative medicine
- biocompatible materials for sustainable environment

- improvements in human capabilities, societal outcomes, and the quality of life

- attention to ethical issues and societal needs



Nanobiomedicine

- applies nanoscale principles and techniques to understanding and transforming inert materials and biosystems (non-living, living or thinking) for medical purposes such as drug synthesis, brain understanding, body part replacement, visualization, and tools for medical interventions
- better understanding of living systems and for developing new tools for medicine and solutions for health care
- understanding the processes inside cells and neural systems

Molecular medicine

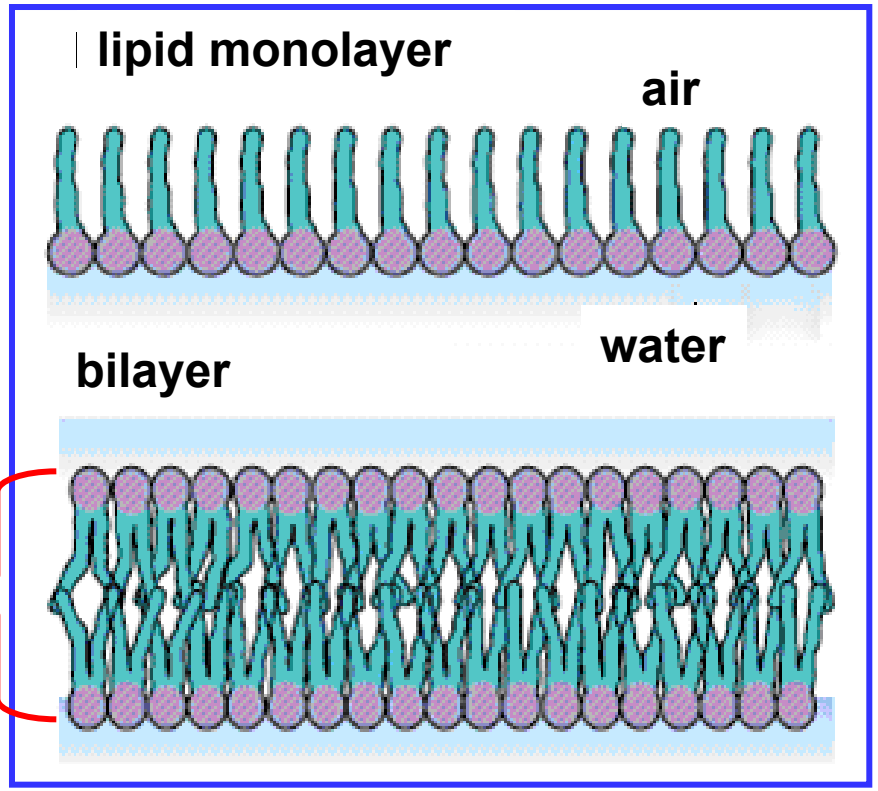
- **nanotechnology provides research tools and technology platforms for biomedicine**
  - examples include working in the subcellular environment
  - investigating and transforming nanobiosystems (e.g. nervous system) rather than individual nanocomponents
  - developing new nanobiosensor platforms
- **methods of nanotechnology help to understand fundamental biological processes**
  - self-assembling, subcellular processes, and systems biology
- **cell as a highly organized molecular mechanism**
  - abilities of information utilization, self-organization, self-repair, and self-replication
- **single molecule measurements**
  - dynamic and mechanistic properties of molecular biomachines, both in vivo and in vitro
  - direct investigation of molecular motors, enzyme reactions, protein dynamics, DNA transcription, and cell signaling

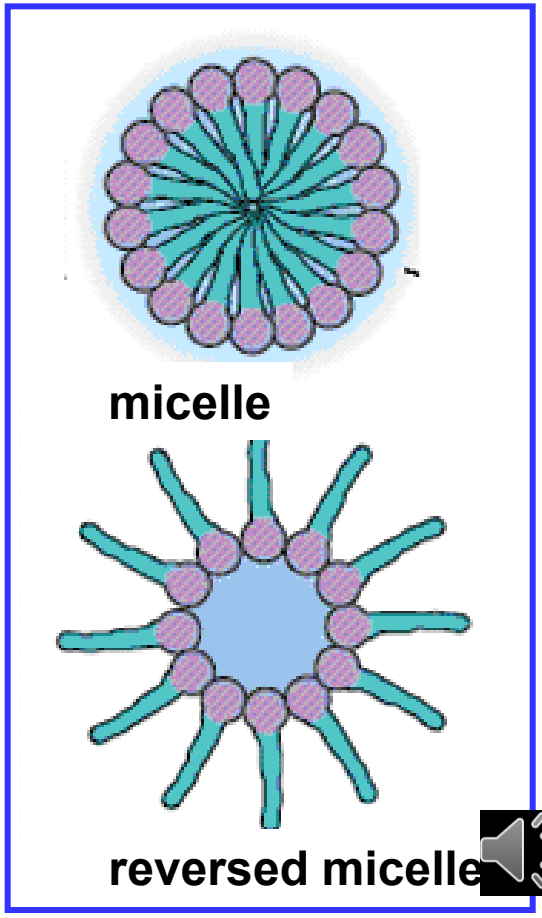
Nanotechnology in drug delivery

- **releasing, targeting, and controlled membrane transport**
- **towards an ideal drug without any side effects:**
  - raise concentration up to the efficient level immediately after the dose is given
  - hold the level for a constant period to allow the drug to do its work
  - return to the original level soon after the treatment period (no interference with the subsequent dose)
- **controlled-release technologies:**
  - **pulse** - a constant amount of drug at a constant time interval
  - **feedback** - released on command from a physical signal
  - **constant rate** release
- **targeting technologies:**
  - active type utilizes a signal peptide, antigen–antibody, receptor-ligand
  - passive - enhanced permeation and retention (EPR) effect near a malignant tumor organ
- **membrane transport:**
  - easily membrane-transfering pro-drug is activated to drug after crossing the membrane

Biomembrane layers

- **lipid bilayer consists of two adhered monolayers, the non-polar parts of the amphiphilic molecules are oriented to the interior, where they interact**
  - all natural biomembrane-based cellular structures
- **micelles are based on a monolayer with globular conformation**
  - in water, nepolar parts are inside
  - reversed micels are formed in non-polar solvents

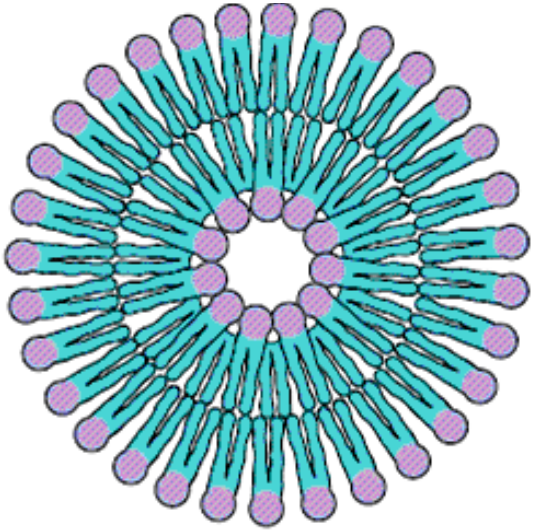


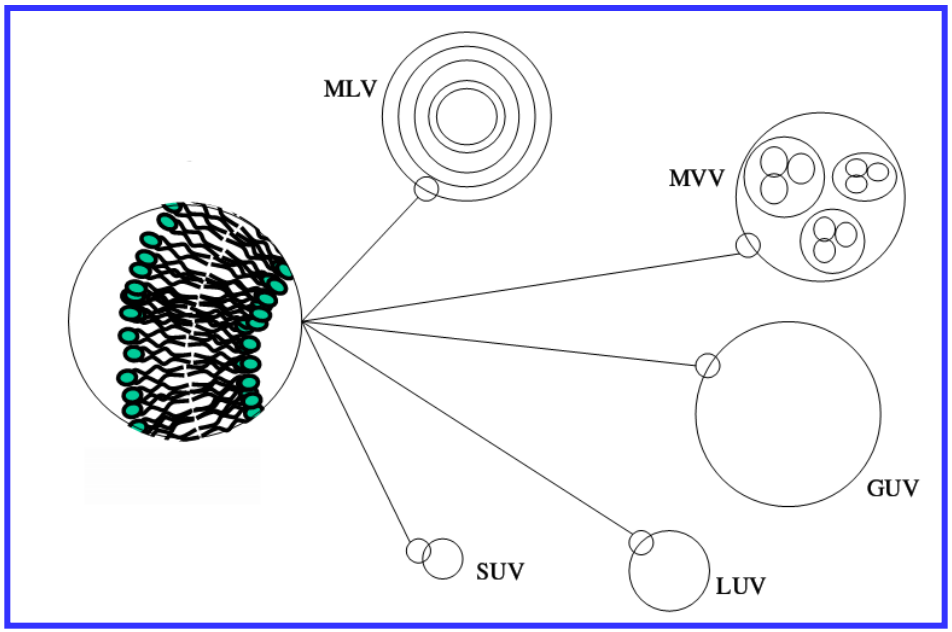


Closed structures - liposomes

- **globular arrangement of the bilayer provides vesicles - liposomes, containing inside acqueous phase**
- **unilamellar (ULV) most suitable for practical use**
  - 20 to 50 nm – SUV, small unilamellar vesicles, high surface curvature
  - 100 nm to around 1 μm LUV, large …“
  - 1 to 200 μm GUV, giant …“
- **multilamellar (MLV, simplest preparation, highest stability), up to 20 μm, concentric / non-concentric arrangements**
- **multivesicular (MVV) – several vesicles contained inside**

liposome – lipid vesicle

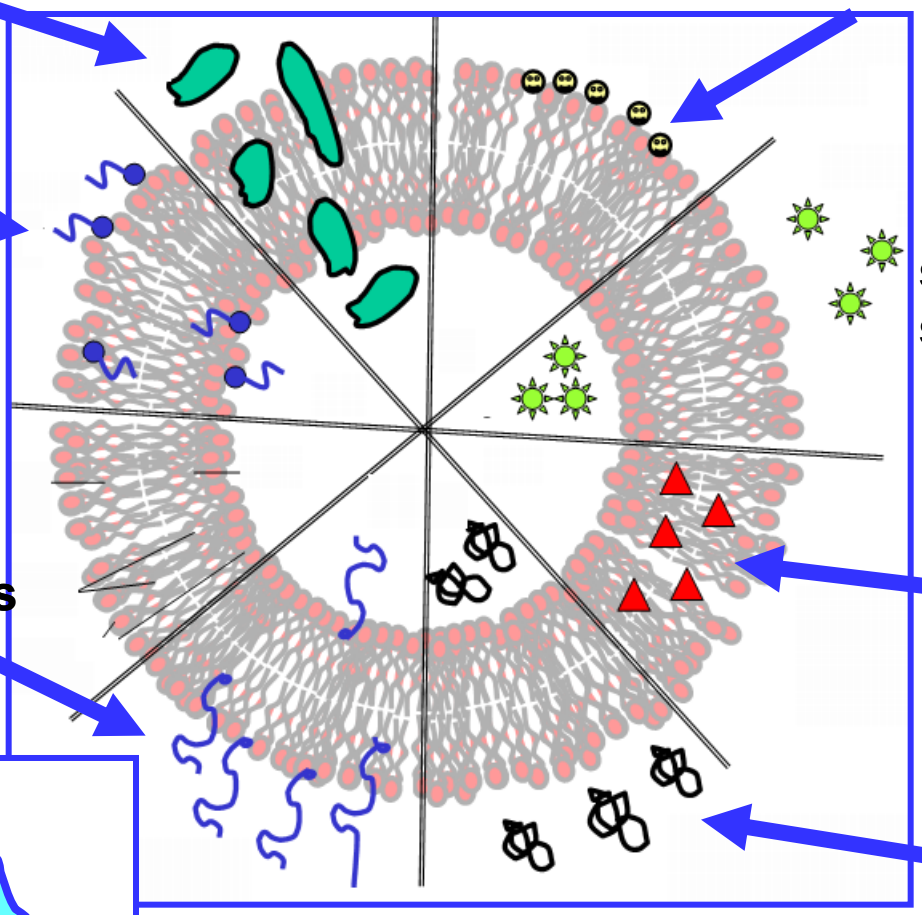




Funcionalized liposomes – targeted delivery

- interacting macromolecules
- amphiphilic ones
- anchored biomacromolecules

surface anchored molecules

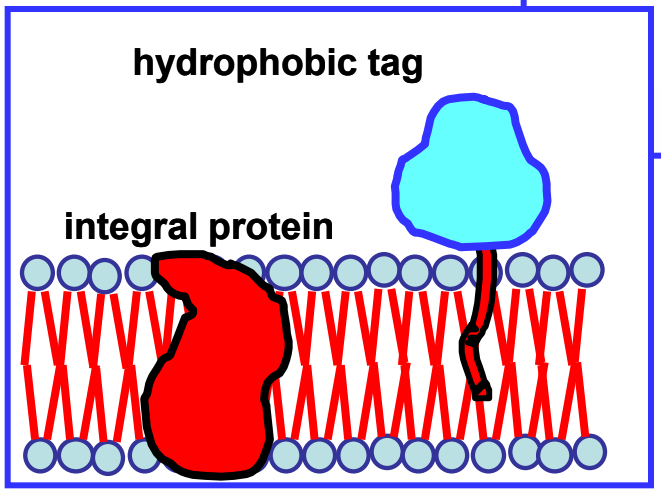


small water soluble substances

lipophilic substances

water soluble macromolecules

transported substances inside

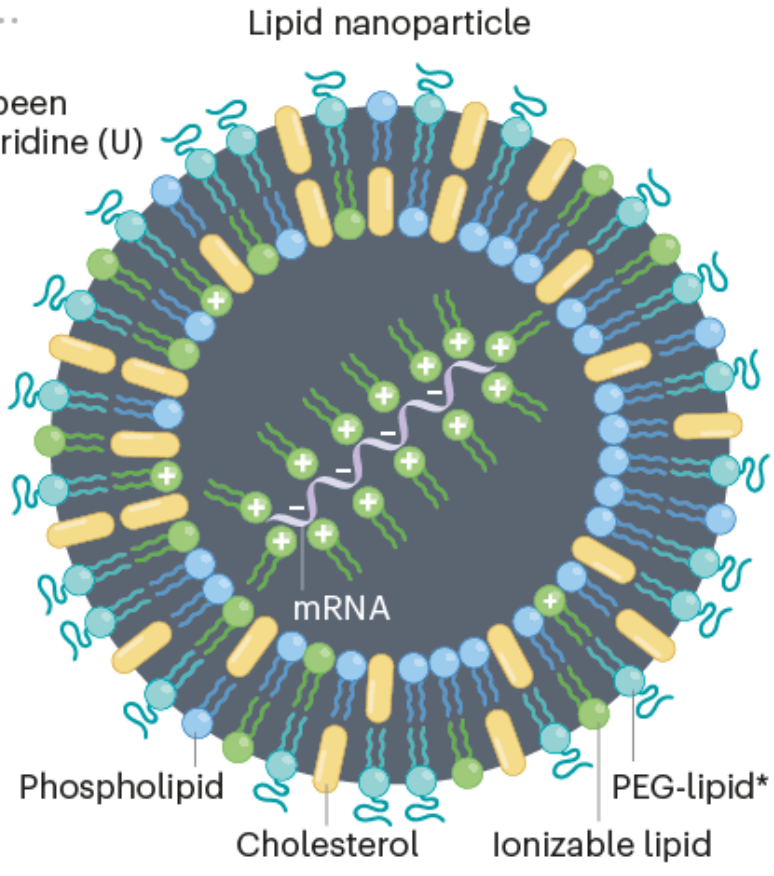


Covid mRNA vaccine

COVID-19 vaccines made from messenger RNA use lipid nanoparticles — bubbles of fats — to carry the molecules into cells. The mRNA contains the code for cells to produce the 'spike' protein that the coronavirus SARS-CoV-2 uses to enter cells. Here are key innovations in the design of these vaccines.

The vaccines made by Moderna and Pfizer–BioNTech use mRNA that has been chemically modified to replace the uridine (U) nucleotide with pseudouridine (Ψ). This change is thought to stop the immune system reacting to the introduced mRNA.

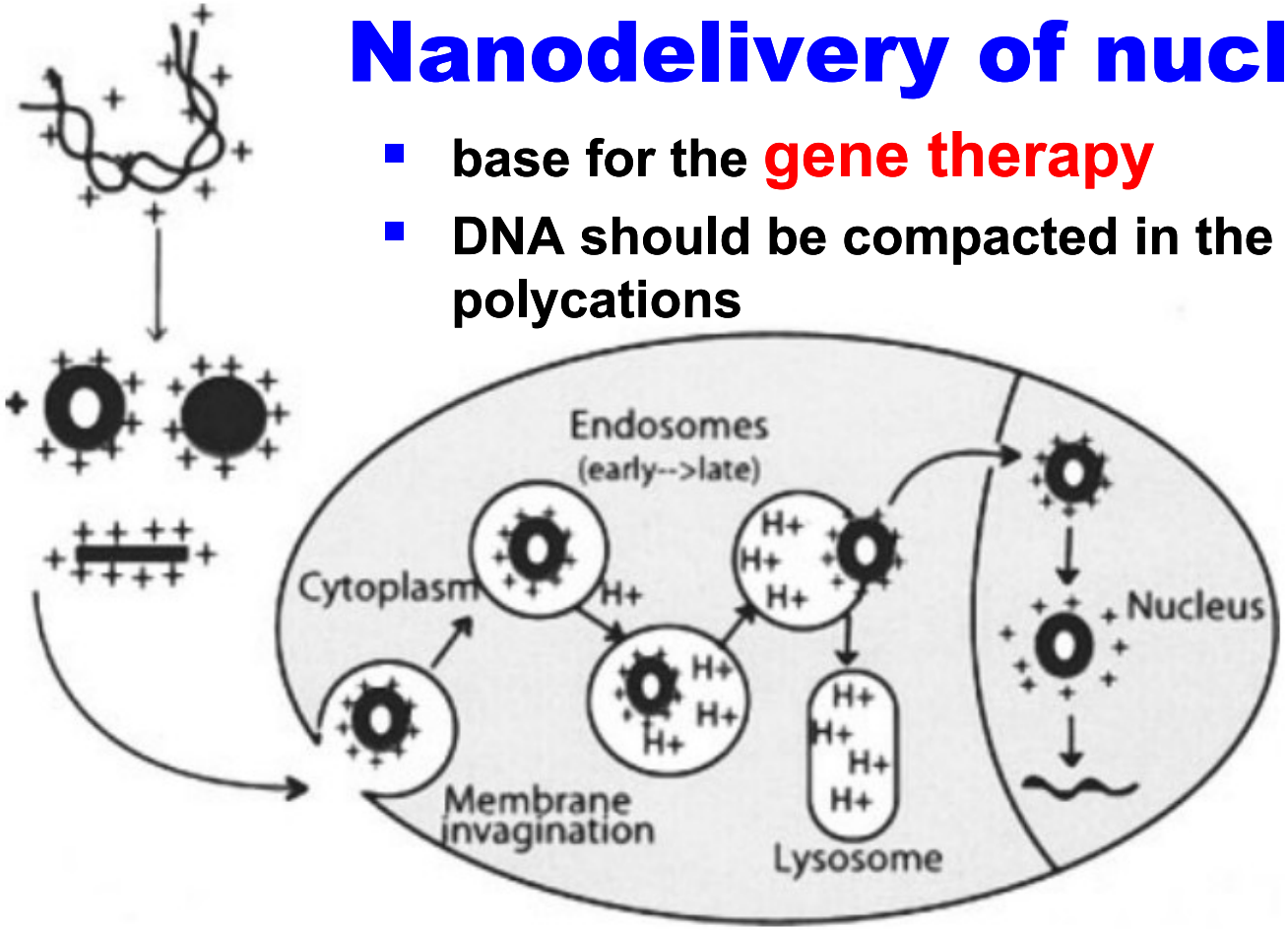
To help the body mount an effective immune response to later SARS-CoV-2 infections, the mRNA sequence is adapted to stabilize the spike protein in the shape it uses when fusing with human cells.



The fatty nanoparticle around the mRNA is made of four types of lipid molecule. One of these is 'ionizable': in the vaccine, many of these molecules have a positive charge and cling to negatively charged mRNA, but they lose that charge in the more alkaline conditions of the bloodstream, reducing toxicity in the body.

Nanodelivery of nucleic acids

- base for the gene therapy
- DNA should be compacted in the presence of polycations

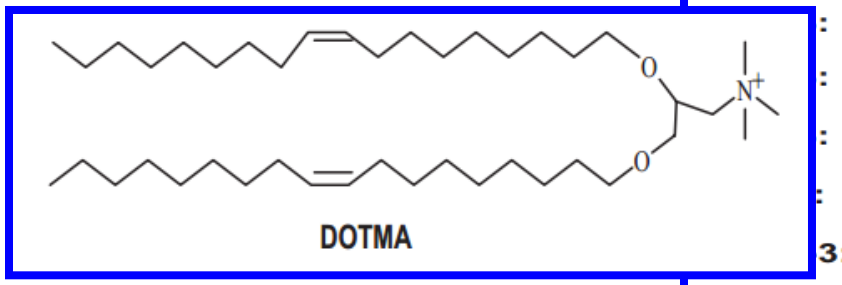


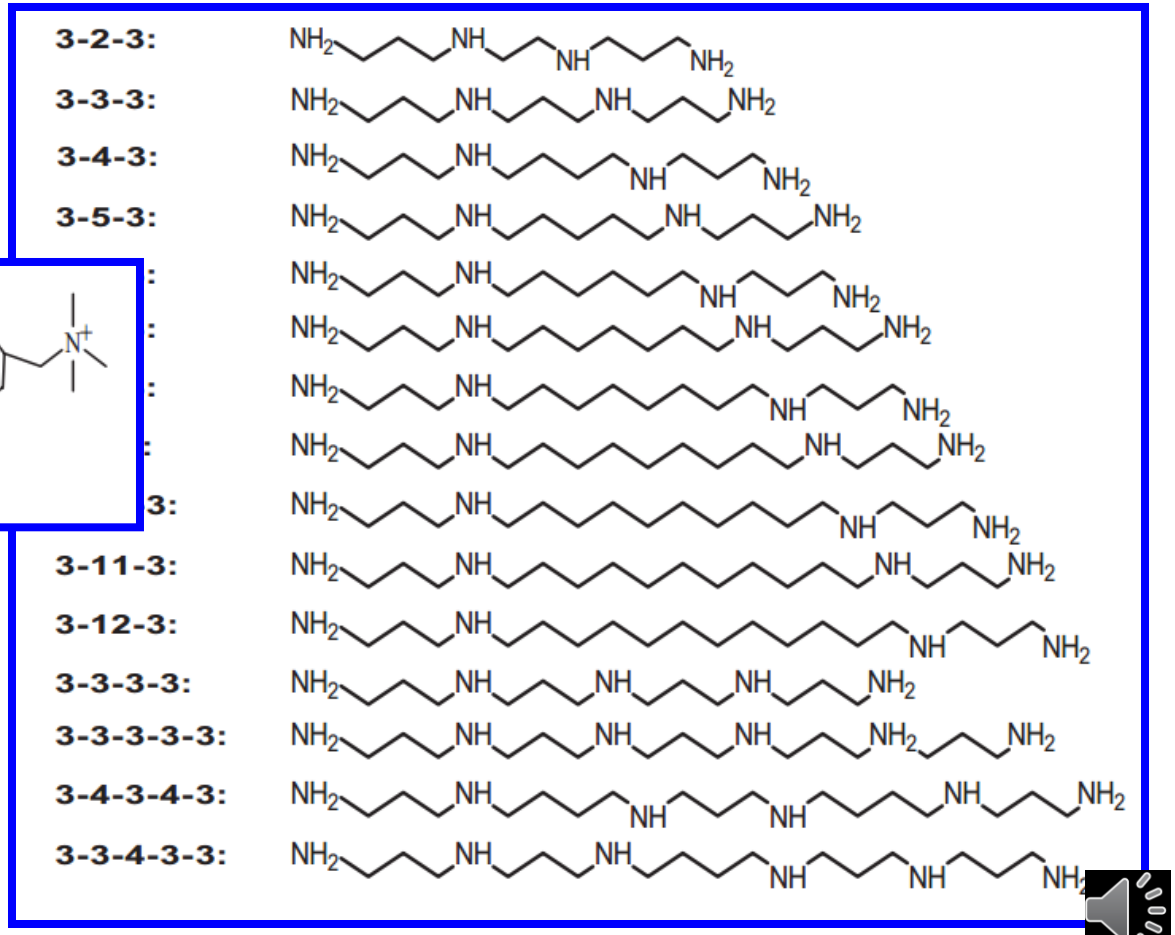
- ordered structures - toroids, rods, and spheroids

- Interact with anionic proteoglycans, transported by endocytosis
- accumulate in the acidic vesicles and raise the pH of the endosomes, inhibiting the degradation of DNA
- protein influx destabilizes the endosome and releases DNA
- translocated to nucleus through pore or with the aid of nuclear localization signals
- decondenses

DNA NPs

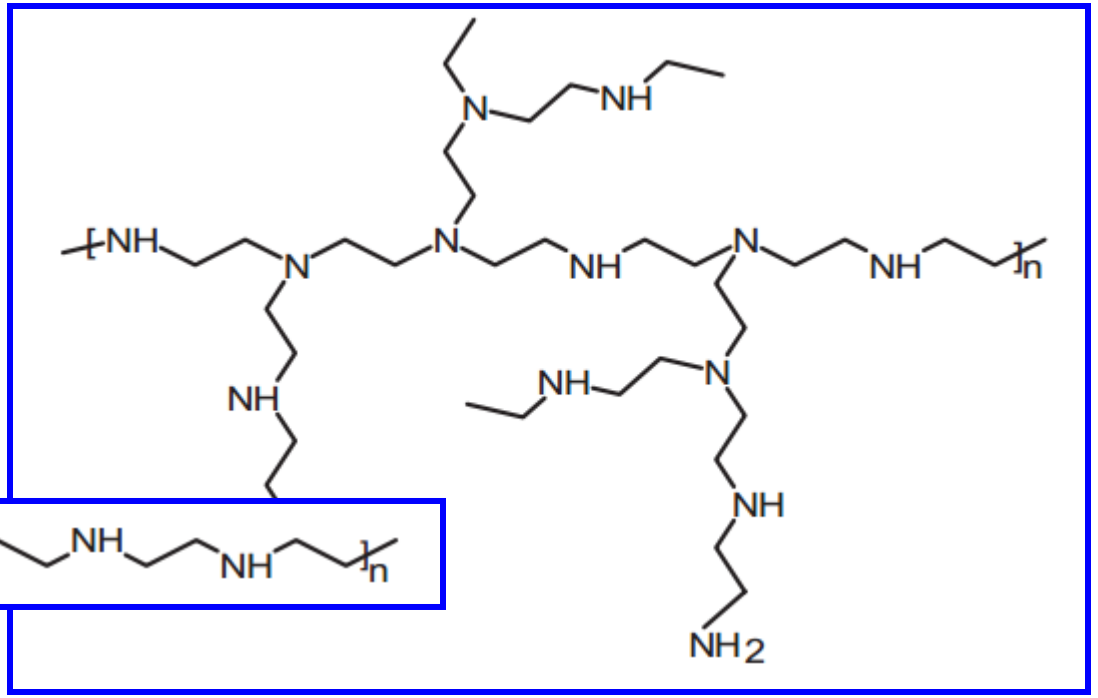
- polyamines spermidine and spermine (3-4-3) and their synthetic analogues are excellent promoters of DNA nanoparticle formation
- **polyamines**
- cationic lipids

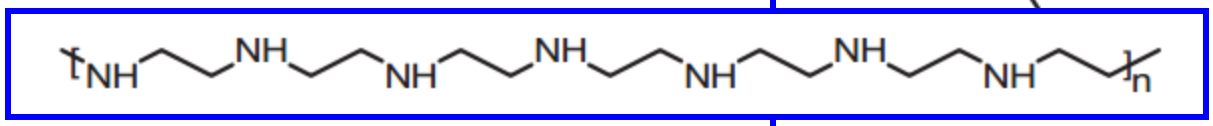




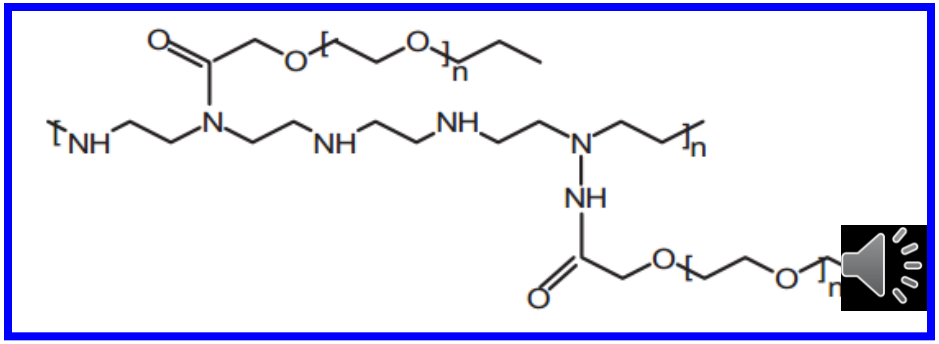
PEI

- polyethylene imines
- linear / branched



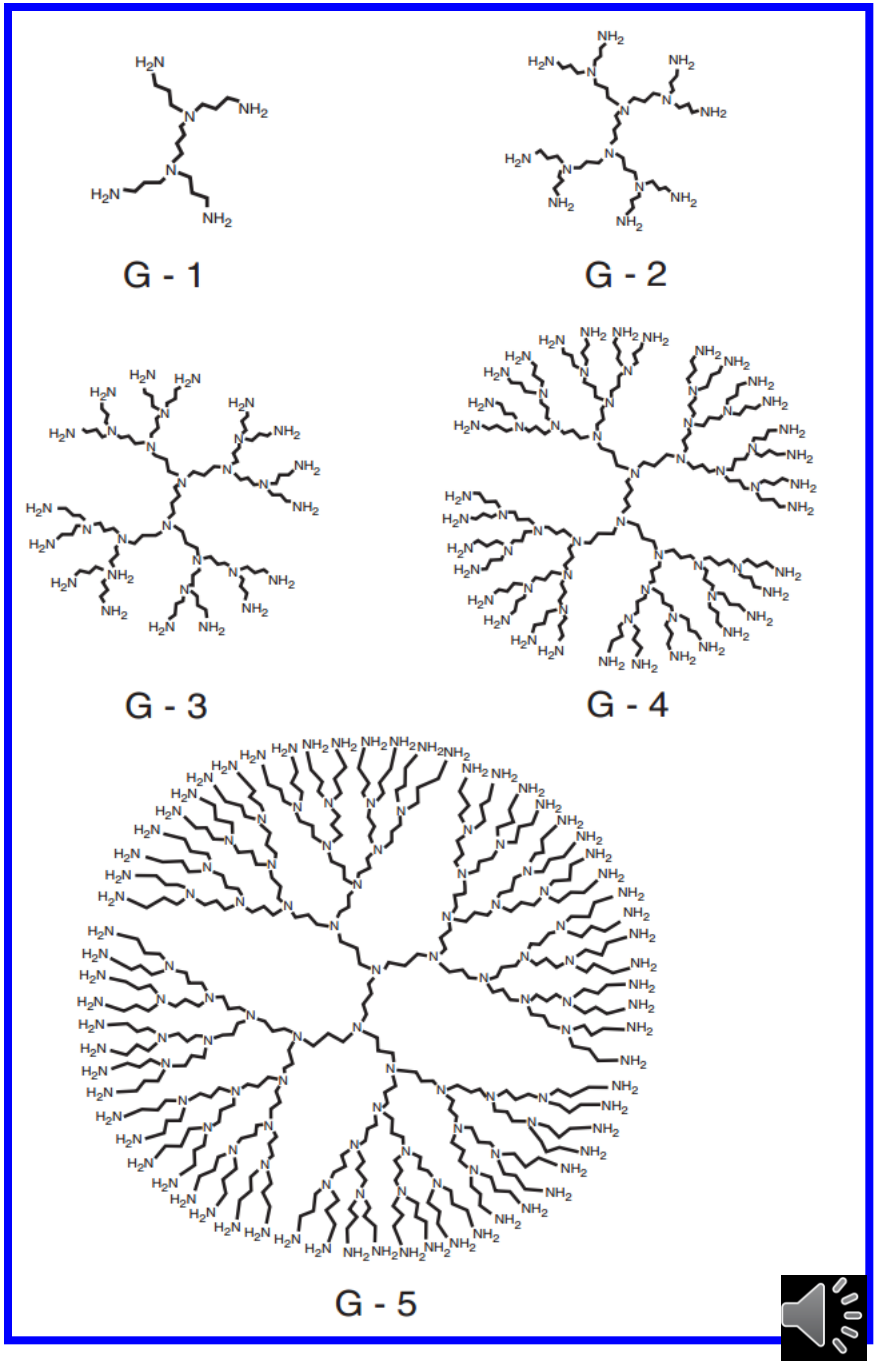


- induces condensation of DNA to nanoparticles
- branched PEI acts as a proton sponge at the endosomal pH
- buffering capacity of PEI is believed to contribute to its ability to deliver DNA within cells without degradation
- PEGylation improves the stability of nanoparticles and increases their in vivo circulation time

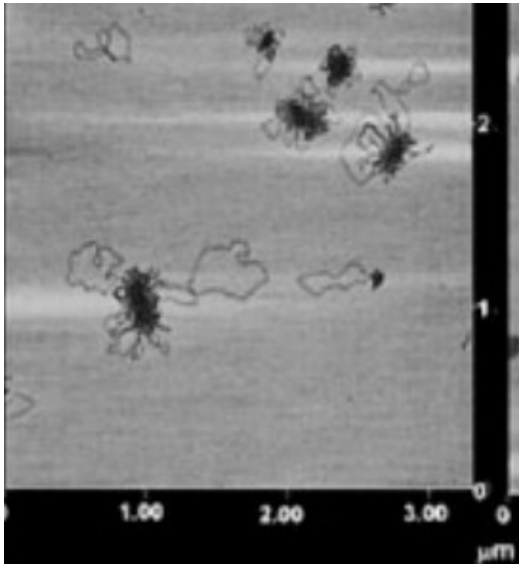


Dendrimers

- polyamidoamine (PAMAM) and polypropylenimine (PPI) dendrimers - ability to provoke DNA nanoparticle formation and facilitate DNA transport
- monodispersity and controllable surface functionality are advantages
- five generations of polypropylene imine dendrimers:

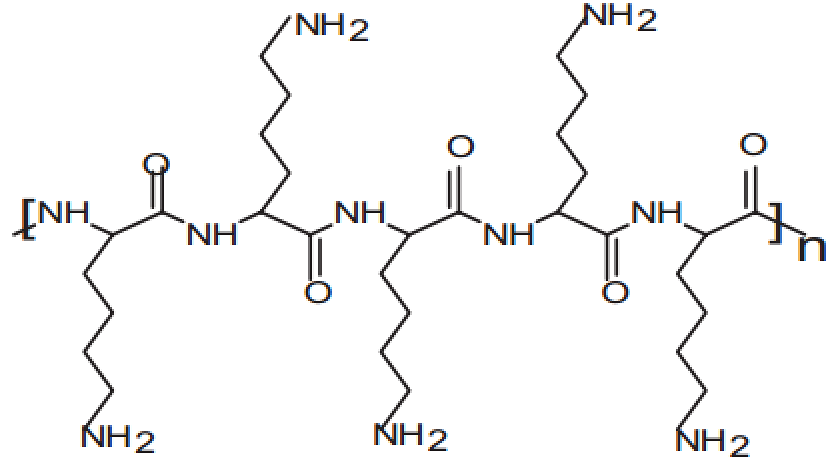


- DNA with dendrimers
- (AFM)

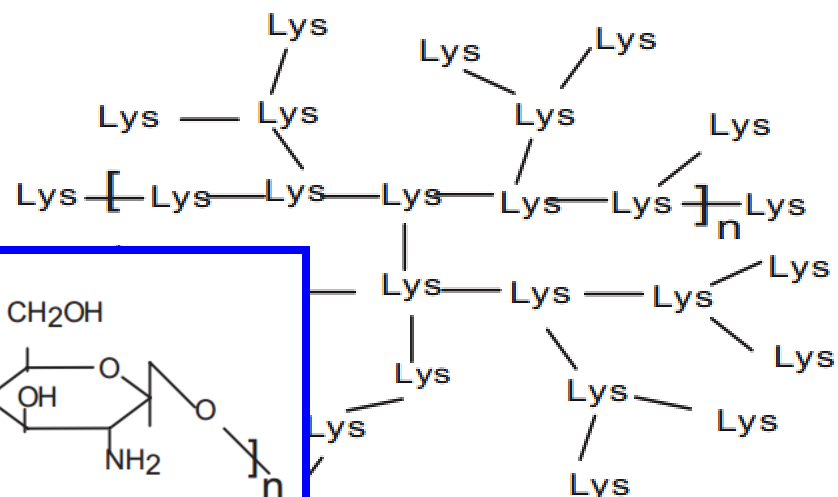


Peptides

- native – cationic histones and protamines
- polylysines

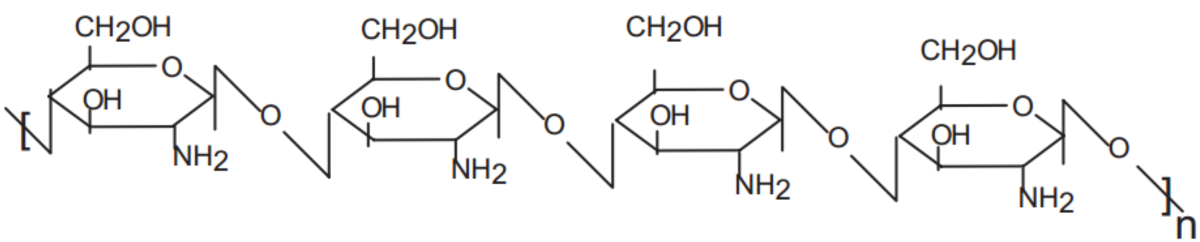


Poly-L-lysine (linear)



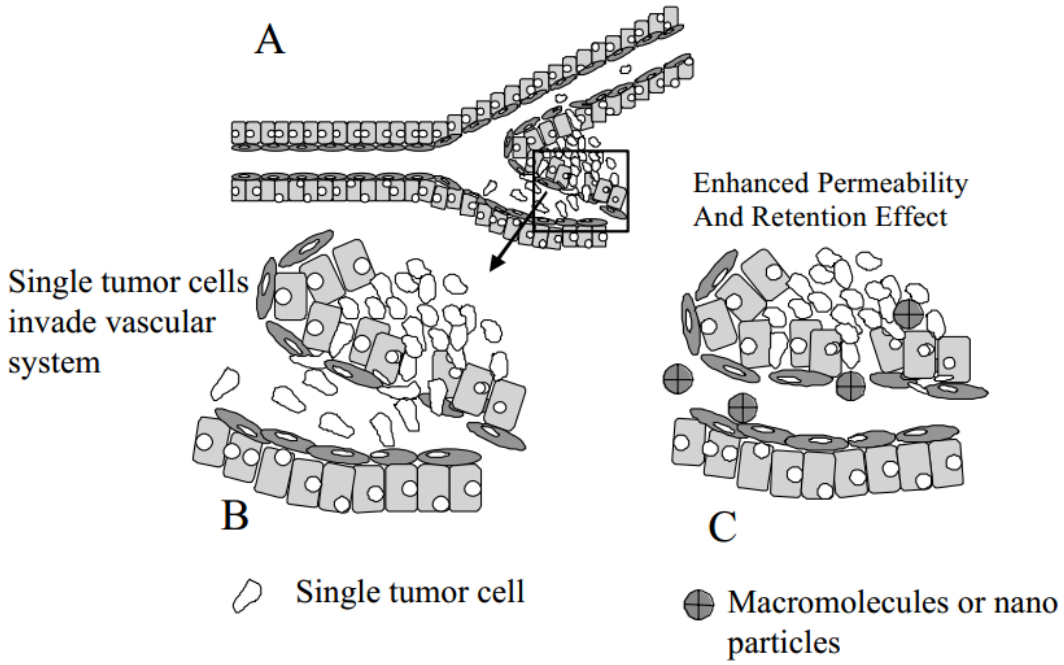
Poly-L-lysine (branched)

Chitosan



Tumor tissues

- various structures and areas, actual cancer cells can occupy <50%, vasculature 1–10%, remaining structure consists of a collagen-rich matrix
- tumor blood vessels differ from normal vasculature in being up to 3–10 times more permeable



- drugs are transported into the tumor cells through interendothelial junctions and vesicular vacuolar organelles and fenestrations
- pore cutoff size in tumor tissue is between 100 and 780 nm

Cells can make incredible journeys—sometimes even traversing the entire body. They navigate by chemotaxis: moving based on changing concentrations of particular chemicals that point the way to the target area. But this kind of gradient cannot extend for long distances without trailing off, so scientists have wondered what guides cells over the chemical hills and valleys of a longer trip. Understanding the process could someday help researchers better predict how cancer will spread throughout the body or explain how cells get to the right places in a developing embryo.

Cancer cells may forge their own chemical paths to navigate long distances. (Pictured are pancreatic cancer cells.)

Drug delivery to tumors

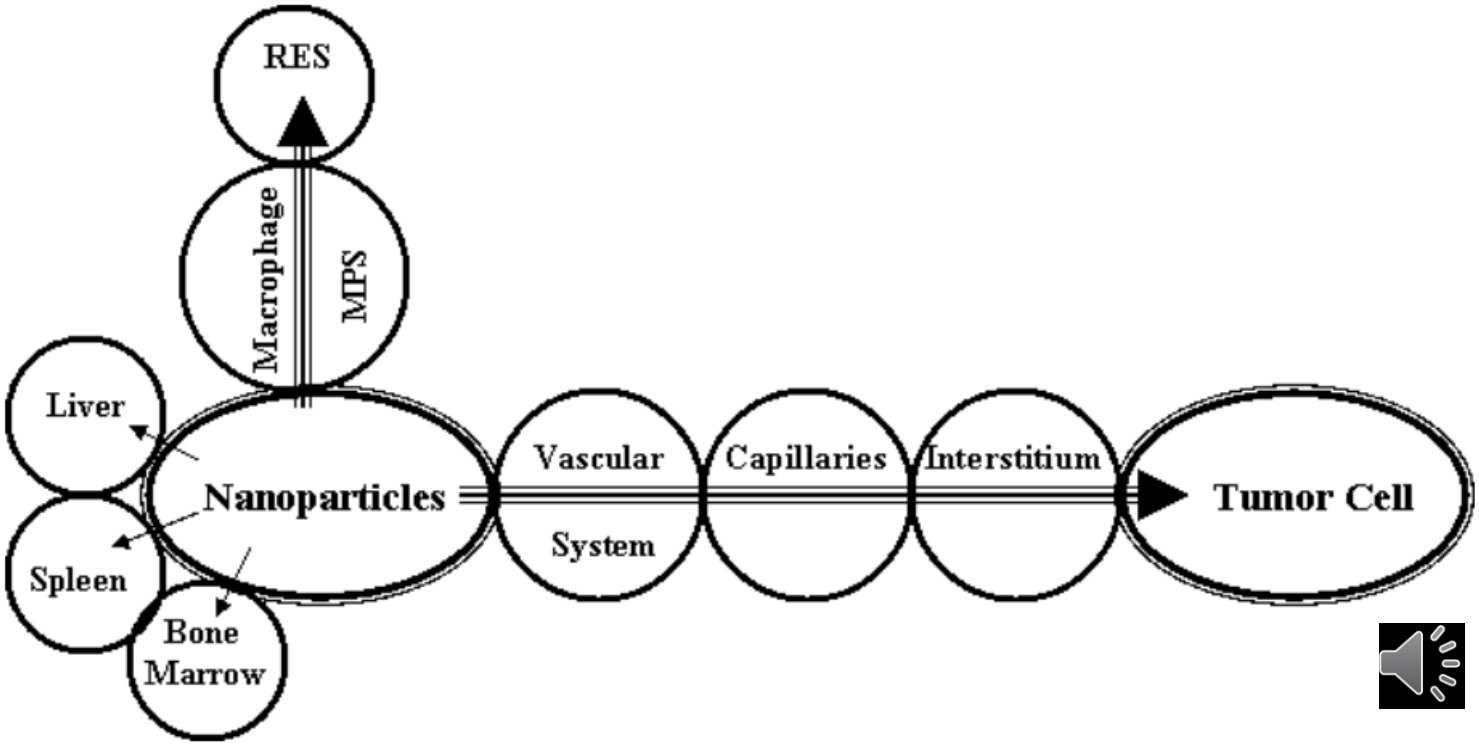
- interstitial compartment of a tumor contains a network of collagen and elastic fiber, which is immersed by hyaluronate and proteoglycan-containing fluid
- interstitial pressure within the tumor tissue is elevated due to the lack of a lymphatic drainage system
- transport of a drug into the tumor area is dependent on the interstitial pressure as well on its composition, charge, and the characteristics of the drug
- colloidal particles larger than 50 kDa enter the interstitial compartment through leaky vessels and accumulate in tumor tissue
- enhanced permeability and retention (EPR) effect

Help of NPs

- deliver the pharmacologically required drug concentration
- increase drug concentration at the target site through extended or controlled release
- overcome multidrug resistance
- limit side effects to vital organs by reducing systemic exposure
- avoid immune response and hematopoietic toxicity
- destroy malignant cells specifically, sparing normal cells
- kill primary tumors inaccessible to surgery
- destroy seeded cancer and dormant cells and metastases
- protect the active drug from alteration and inactivation
- detect cancers at a early stage

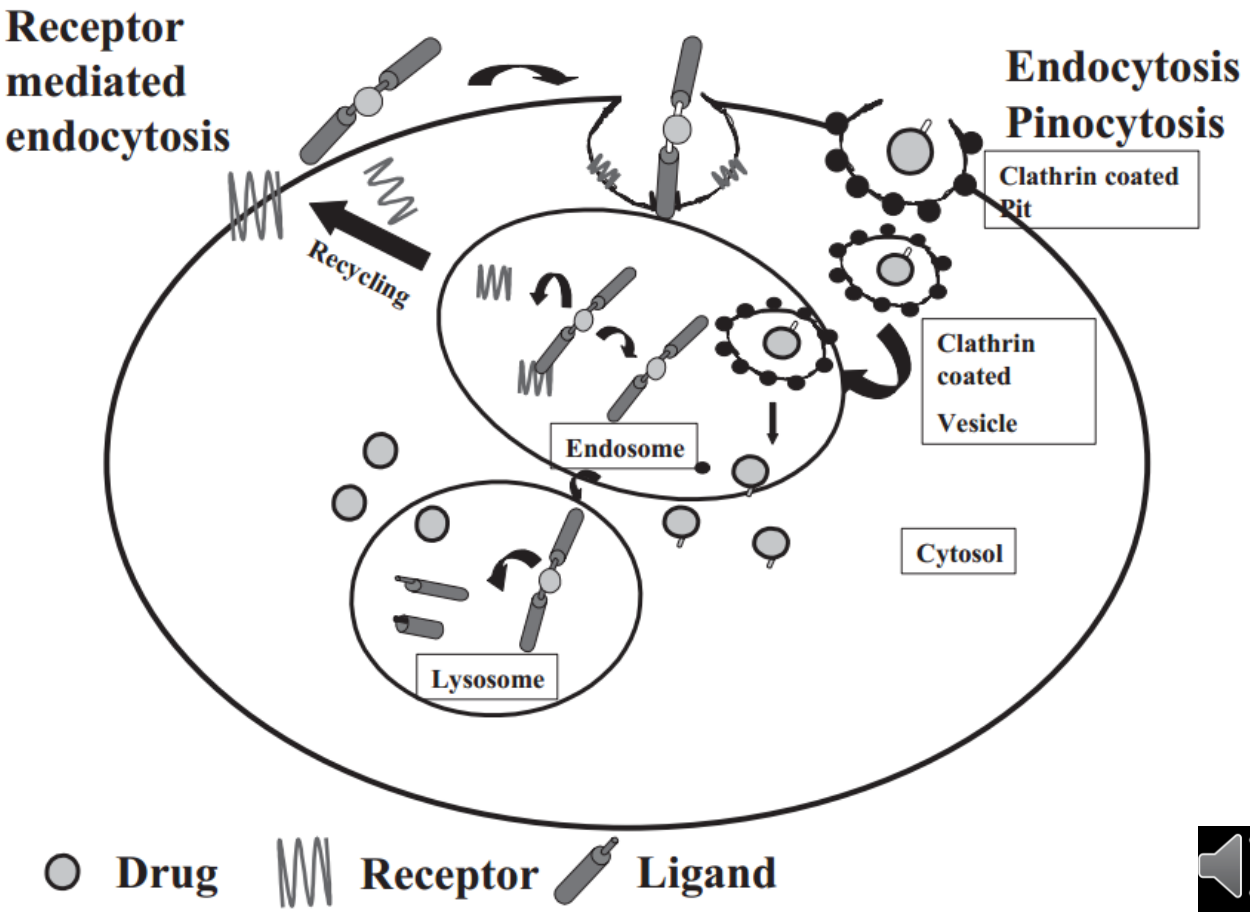
Pathways of nanoparticles

- distribution and routes of NPs after injection
- coating with plasma proteins – opsonization
- macrophages internalize the opsonized nanoparticles through phagocytosis and deliver them to the liver, spleen, kidney, lymph node, and bone marrow
- this clearance can occur within 0.5–5 min
- prevention – coating, size under 100 nm



Cellular uptake

- **pinocytosis, endocytosis, receptor-mediated endocytosis**
- **some NPs can escape lysosomal degradation**
  - important for DNA and macromolecules



Surface treatment or coating of NPs

- **coating with biodegradable matrices – to become "invisible" to macrophages**
- **choice of hydrophilic or hydrophobic matrices for coating determines the fate of NPs**
- **hydrophilic coating prevents interaction with macrophages of the RES, reduces their removal from the circulation, and increases their circulation half-life**
  - dextran, PEG, polyethylene oxide (PEO), poloxamers and poloxamines, silicones
- **hydrophobic coatings are applied to increase opsonization, leading to copious interaction with macrophages, and the nanoparticles are therefore rapidly removed from the circulation**
  - this approach is applied for targeted delivery of nanoparticles to the RES of liver and spleen

Polymers for encapsulation

- two different approaches
- 1) as a drug reservoir, which consists of an oily core as vehicle, which carries the drug, and a polymeric outer core layer with a coating
- 2) particles are nanospheres in which the drug is dispersed in a polymeric matrix

- synthetic biodegradable polymers such polyvinylpyrrolidone (PVP), chitosan, polyalkylcyanoacrylates and
- polylactides such as polyisohexylcyanoacrylate (PIHCA), polyethylcyanoacrylate, and polyisobutylcyanoacrylate (PIBCA)
- PLGA (FDA approved) degrades slowly, releasing the drug, and is therefore used for controlled release

Site-specific delivery

- passive targeting includes manipulation of the size and/or hydrophobicity or other physicochemical characteristics and can be applied to target the RES

- active targeting involves the direction of magnetic particles by using an external magnetic field or by using ligand-conjugated nanoparticles
- folate-coated NPs specifically target folate receptor

Release

- sustained release through polymer degradation
- enzymatically controlled release
- controlled release through use of thermosensitive polymers
- photochemically controlled release
- pH-responsive release systems
- laser-induced breakdown (LIB)
- ultrasound-mediated release

Implants, prostheses

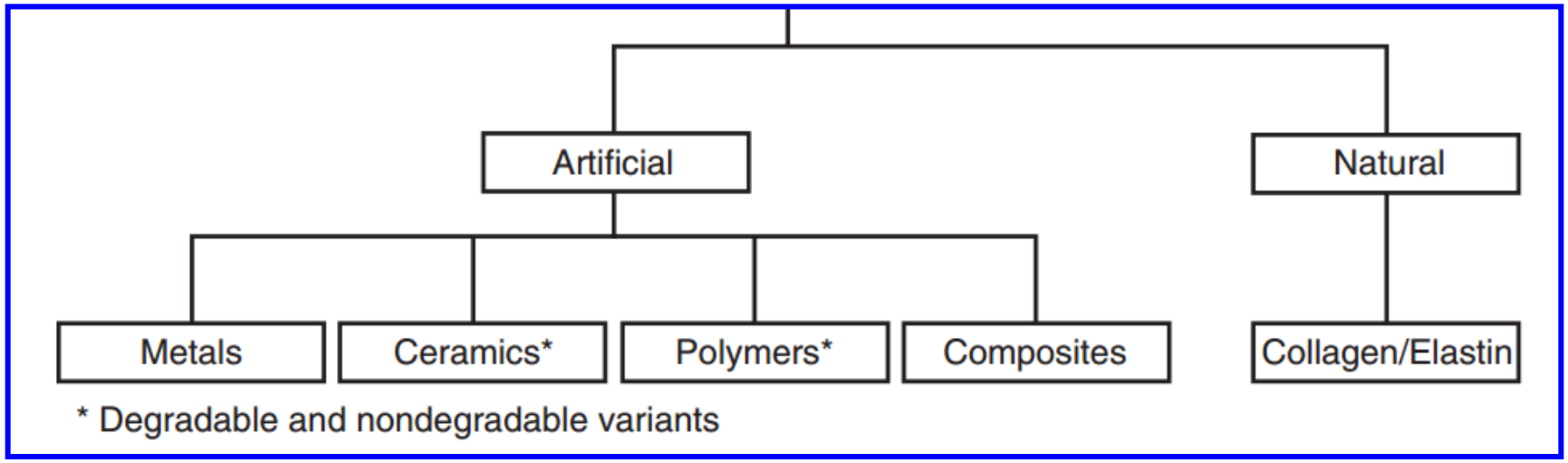
- **increasing life expectancy - growing number of synthetic devices to overcome the problems associated with deteriorating or failing body parts**
- **examples of implants are orthopedic joint prostheses, cardiovascular devices, dental implants, for aesthetic reasons**
- **from biomaterials that have a common property: biocompatibility**
- **this might not be universal for particular type of material – significant effect of location in body and intended function of the device**
  - resistance to adhesion of biomolecules and cells =
  - is biocompatible for the production of cardiovascular devices
  - completely different if used to manufacture artificial joints
  - anyway, small side effects due to “symbiosis” might always occur

Synthetic and natural materials in reparative medicine

Material	Application	Tissue Response
Titanium and its alloys	Joint prostheses, oral implants, fixation plates, pacemakers, heart valves	Inert
CaP ceramic	Joint prostheses, oral implants, bone replacement, middle ear replacement	Bioactive
Alumina	Joint prostheses, oral implants	Inert
Carbon	Heart valves	Inert
PTFE	Joint prostheses, tendon and ligament replacement, artificial blood vessels, heart valves	Inert
Poly(methylmethacrylate)	Eye lenses, bone cement	Tolerant
Poly(dimethylsiloxane)	Breast prostheses, catheters, facial reconstruction, tympanic tubes	Unknown
Poly(urethane)	Breast prostheses, artificial blood vessels, skin replacements	Inert
PLA	Bone fixation plates, bone screws	Inert
PGA	Sutures, tissue membranes	Inert

PTFE = poly(tetrafluoroethylene). PLA = poly(lactic) acid. PGA = poly(glycolic) acid.

Biomaterials



- **bulk properties (together with the design) determine the strength (mechanical) of an implant**
  - intrinsic properties: elastic modulus, yield stress, and ultimate stress
  - these parameters determine the stiffness, deformability, and strength
  - fatigue – cyclic stresses (much smaller than ultimate tensile stress)
- **surface properties are important for interactions of implant with biological systems**
  - should not be toxic, carcinogenic, pyrogenic, cytotoxic, or antigenic to living cells
  - surface energy - important factor in the establishment of cell adhes

Biomaterial in the living environment

- interaction of biosystems with biomaterial surfaces can be desirable - enhanced integration in the body
- non-interacting surfaces – when deposition of biological material (biofouling or bioadhesion) is undesirable
- control over bioadhesion - encourage adhesion of host cells but discourage adhesion of infectious bacteria - a common cause of implant failure
- biodegradation - production of polymeric and ceramic biomaterials whose degradation rates can be controlled
- devices that in time can be replaced by native tissue

Biological processes

- **implant = intrusion of a foreign object - initiates a response of the body - wound healing**
  - soft x hard tissues (scars)
- **formation of thrombus – incoming fluids and blood, activation of platelets and coagulation cascade, polymerization of fibrin = clot**
  - matrix for future incoming cells
- **inflammation initiated as release of vasodilators, chemoattractants, and other mediators**
  - platelet-derived growth factor (PDGF)
  - tumor growth factor-beta (TGF-β) by platelets
- **activation of the complement cascade within the coagulating fluid surrounding the implant**
- **recruitment of inflammatory and other cells (chemotaxis)**
  - development of new blood vessels (angiogenesis), and overall cell regulation
- **nonspecific defense mechanisms by cells and factors**
  - granulocytes, monocytes, and the complement system
  - resident inflammatory cells (macrophages and mast cells) try to eliminate intruders
  - if necessary, specific immune responses - production of antibodies by B lymphocytes and/or activation of cytotoxic T lymphocytes can be initiated

Reparative phase

- **formation of new tissue requires the activation and/or proliferation of distinct cell types, resulting in the replacement of lost or damaged tissue**

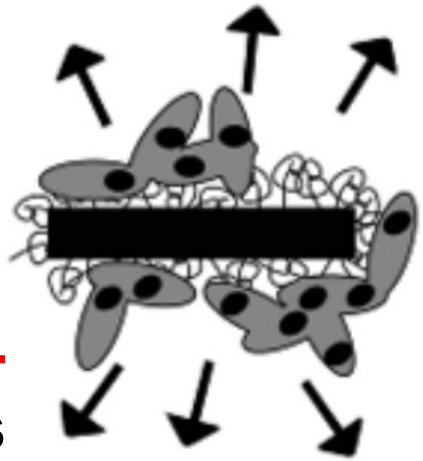
- **soft tissue healing – fibroblasts and endothelial cells**
  - formation of new extracellular matrix, angiogenesis
  - nutrients and oxygen for proliferating cells
- **hard tissue - ossification (bone formation), two ways:**
  - intramembranous ossification is carried out by osteoprogenitor cells present in the cambium layer of the periosteum
  - endochondral ossification occurs at and overlies the defect site and undifferentiated mesenchymal cells attracted from tissues surrounding the defect become committed cartilage-producing cells, mineralization of the cartilage tissue leads to bone formation

Biointerface

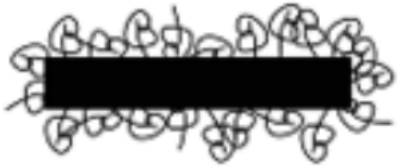
- **initial contact of implant and host relies on non-cellular interactions**
- **newly introduced implant is surrounded by liquid**
  - water mono / multi layer
  - biomaterial surface - hydrophobic or hydrophilic (cell adhesion)
- **ions (Na^+, Cl^-) appear**
- **followed by adsorption of proteins**
  - denaturation might occur
  - these adsorbed modified proteins can be recognized as a foreign material stimulating reactions
- **living cells are coming**
- **final result of implantation:**
  (1) integration, (2) extrusion,
  (3) resorption, (4) encapsulation

Phases of interaction

1 insertion of implant



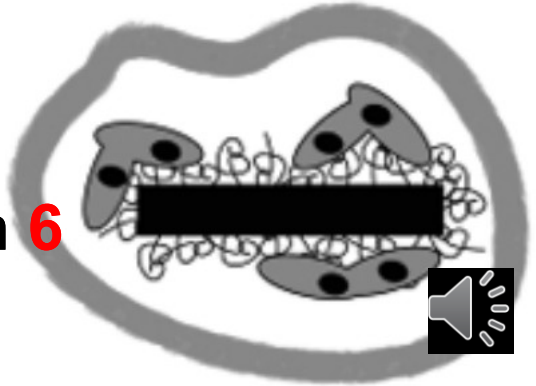
release of attraction 4 factors



2 adsorption of proteins

extracellular matrix 5

3 incoming cells



encapsulation 6

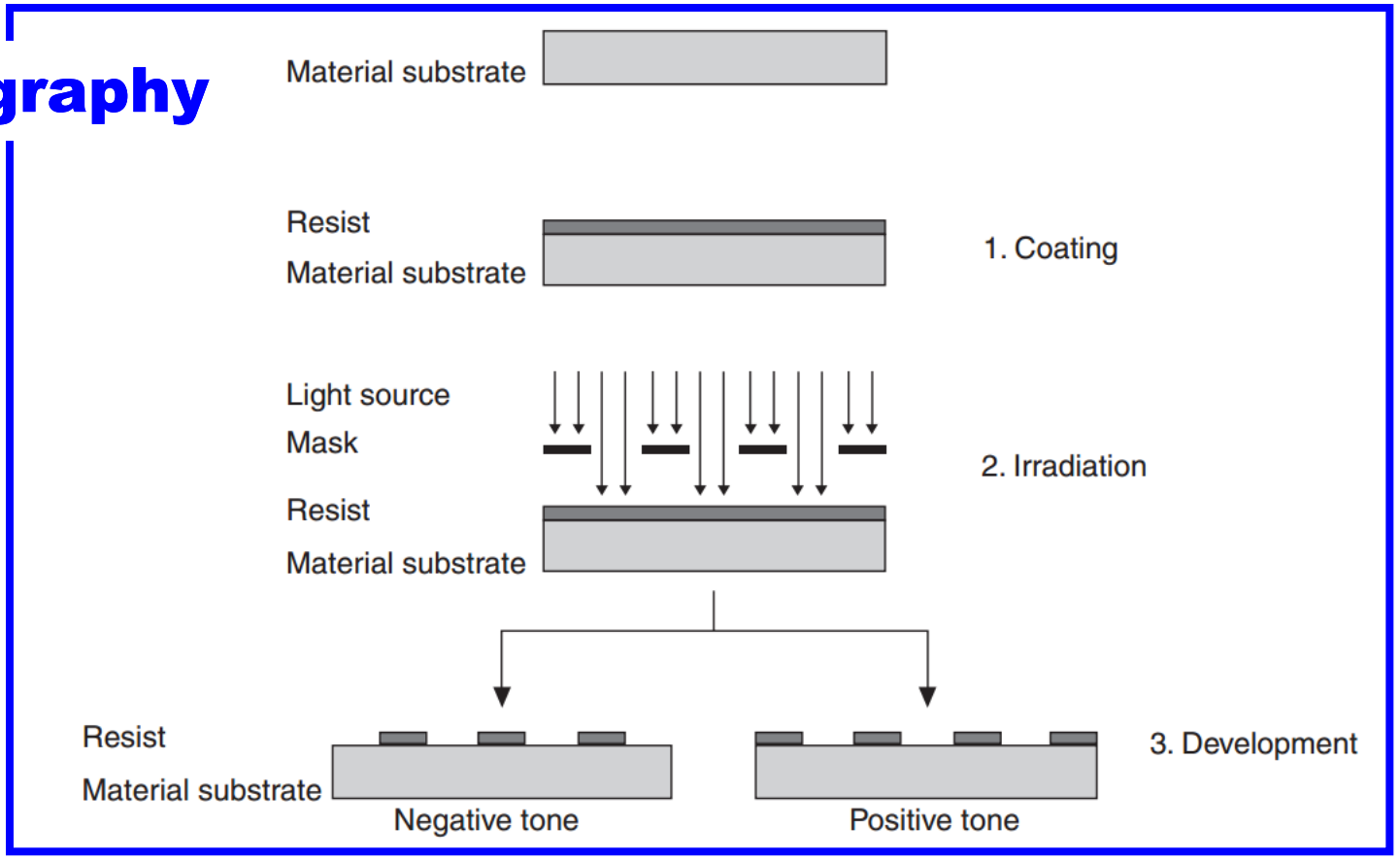
Contribution of “nano”

- making more bio-friendly (biocompatible) surfaces through nanostructuring – contacts for (oriented) adhesion of both extracellular matrix proteins (ECM) and cells

- Nanofabrication methods

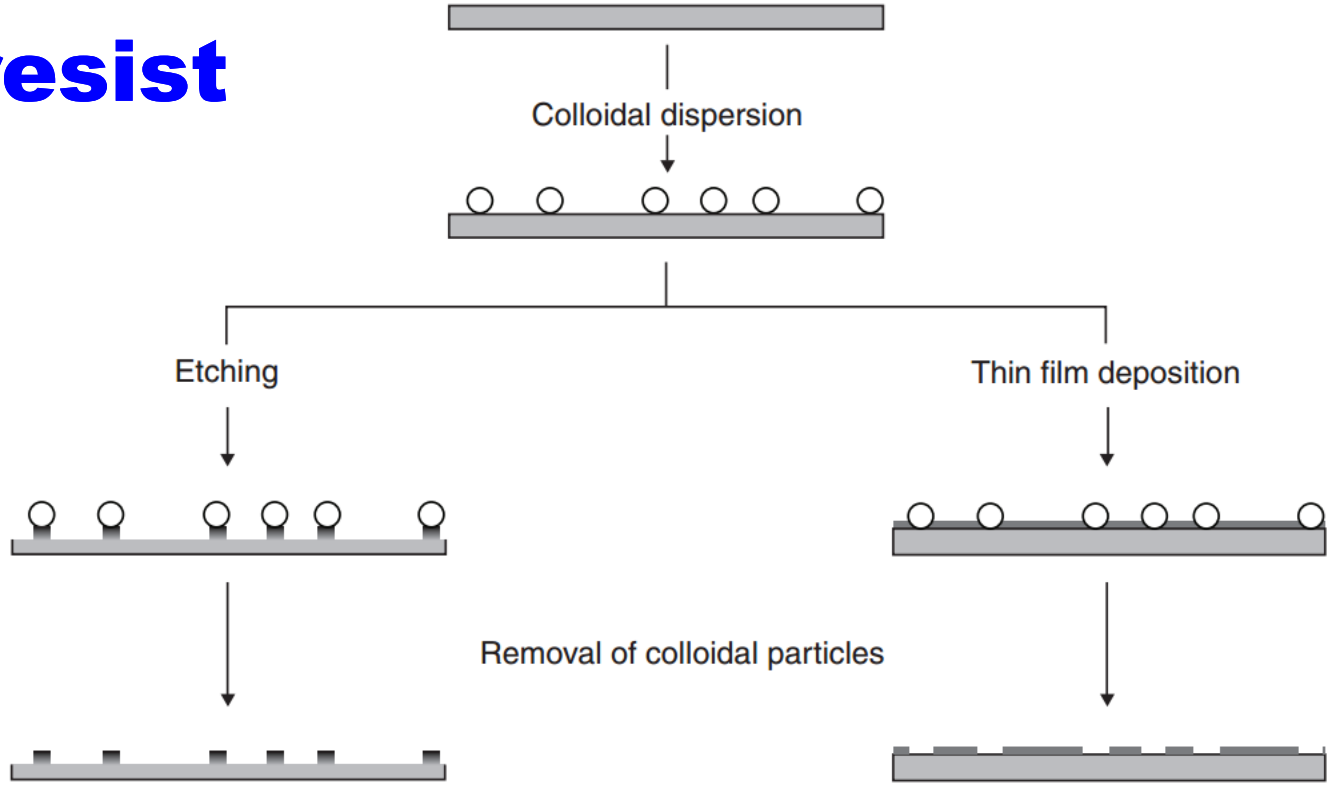
Type of System	Materials	Resolution
Lithography	Silica, silicon, silicon nitride, silicon carbide	x, y, and z to 10 nm
Colloidal resist	Silica, silicon, silicon nitride, silicon carbide	x, y, and z to 5 nm
Self-organizing or self-assembling	Polymer demixing, self-assembling particles and monolayers, other self-assembling systems	In 10-nm range
Soft lithography	Any fairly large molecule	x and y to 200 nm, z to one monolayer
Biomimicry	Many	Actual native dimensions

Photolithography



- **etching** - pits, grooves, and other topographies of controlled shape and size
- **deposition** of a thin film – coating the exposed area with a desired solution, from which the solvent evaporates or in which the particles (molecules) organize themselves in a specific conformation (selfassembly)

Colloidal resist

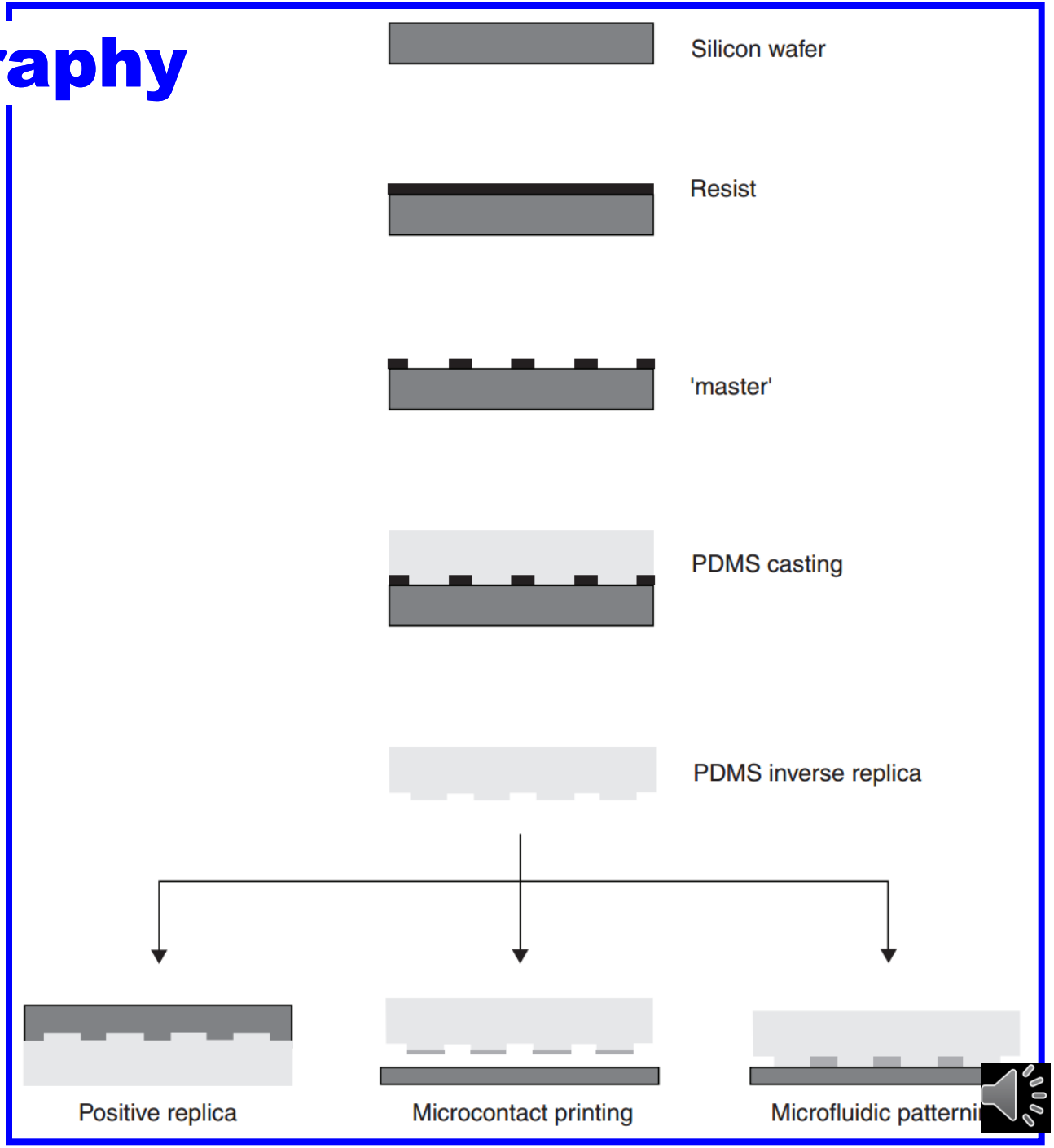


- colloidal particles of different materials and sizes down to 5 nm can be produced and subsequently dispersed over a biomaterial surface
- the adsorbed particles can be used as a template for patterning the underlying surface
- both etching and deposition are feasible
- removal of particles provides the desired pattern

Soft lithography

- **hard** template based on Si
- **soft** template based on elastomer
- pattern transfer to the target

- often combined with SAM



Biomimetic approaches

- **create an implant surface which is not (or to a lesser extent) recognized as foreign by the host**
- **constituents of the natural cellular environment (ECM proteins) can help – domains influence cell behavior**
  - interactions of the receptor family of integrins
  - including proliferation, migration, morphological change, gene expression, and cell survival by intracellular signaling
- **how to achieve:**
  - physical adsorption (van der Waals or electrostatic interactions)
  - physical entrapment (use of a barrier)
  - covalent attachment (also more sophisticated such as covalent linking to polymeric networks)
- **not necessarily complete proteins, significant peptides**
  - Arg-Gly-Asp (RGD) amino acid sequence - cell-binding domain of fibronectin, serves as a ligand for integrin receptor (α5β1)

Calcium phosphates

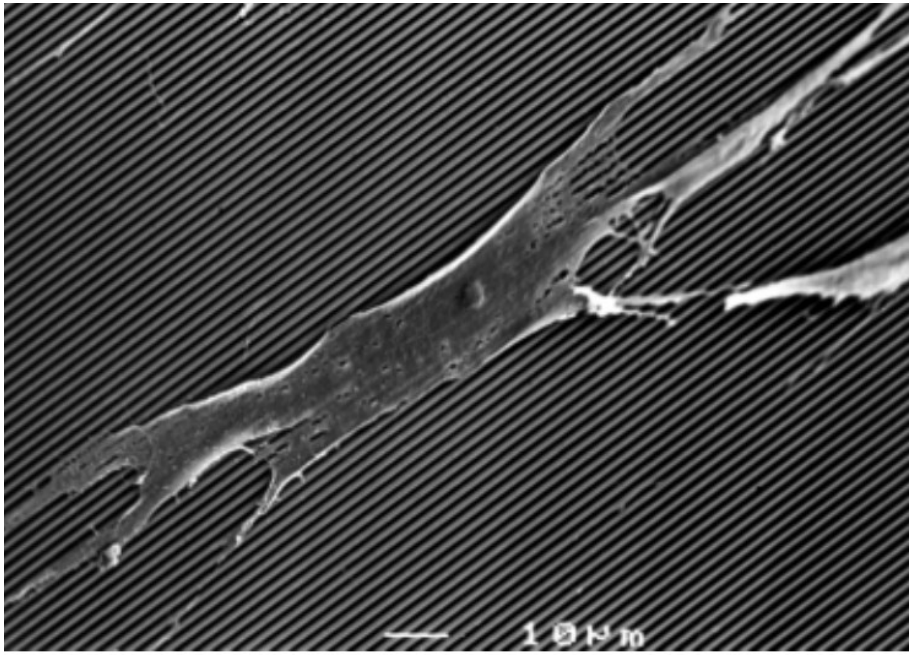
- **natural hard tissues comprise precipitated minerals**
  - also used for biomimetic biomaterial surfaces
- **hydroxyapatite surface coatings for bone implants**
- **Ca phosphates are bioactive - allow dynamic interactions favoring bone formation with implant surroundings**
- **deposition techniques:**
- **magnetron sputtering**
- **plasma spraying**
- **electrostatic spray deposition**

- **generation of nanostructured coatings**

Topographical nanostructures

- **increase in surface area - greater potential for tissue integration (mechanical interlocking, contact guidance)**
- **tested shapes - grooves, pits, ridges, cliffs, tunnels, steps, waves, wells, tubes, nodes, pillars, pores, spheres, cylinders, …**
- **cell responses - orientation, extension, movement, and activation**
  - phosphorylation, actin polymerization, mRNA expression, phagocytic activity
- **control over cellular alignment (including cellular extenstions)**
- **precise effects still rather unclear**

Contact guidance



- **rat dermal fibroblasts on microgrooved substrates**
- **cells become oriented and elongate along the surface grooves**

- **specific role for biomaterial surface nanotopography has been demonstrated for growing nerve tissue**
- **achieved control over the outgrowth of neurites from the cell bodies of neurons**
  - both the sites at which they emerge from cell bodies and directions
  - surface roughness is usually welcome, too

Protein / peptide surfaces

- signalling to cells to modulate spatial behavior
- examples of immobilized peptides

Peptide	ECM Molecule Source	Application
RGD	Multiple ECM molecules, e.g., fibronectin, vitronectin, laminin, collagen, and thrombospondin	Enhance bone and cartilage tissue formation *in vitro* and *in vivo*; regulate neurite outgrowth *in vitro* and *in vivo*; promote myoblast adhesion, proliferation, and differentiation; enhance endothelial cell adhesion and proliferation
IKVAV YIGSR RNIAEIIKDI	Laminin	Regulate neurite outgrowth *in vitro* and *in vivo*
Recombinant fibronectin fragment ($FNIII_{7-10}$)	Fibronectin	Promote formation of focal contacts in preosteoblasts
Ac-GCRDGPQ-GIWGQDRCG	Common MMP substrates, e.g., collagen, fibronectin, and laminin	Encourage cell-mediated proteolytic degradation, remodeling, and bone regeneration *in vivo*

Topography vs. chemistry

- nanotechnological structures:
- isotropic that do not differ chemically from the intrinsic substratum
- anisotropic nanotechnological structures using patterns of molecules chemically different from those of the intrinsic substratum

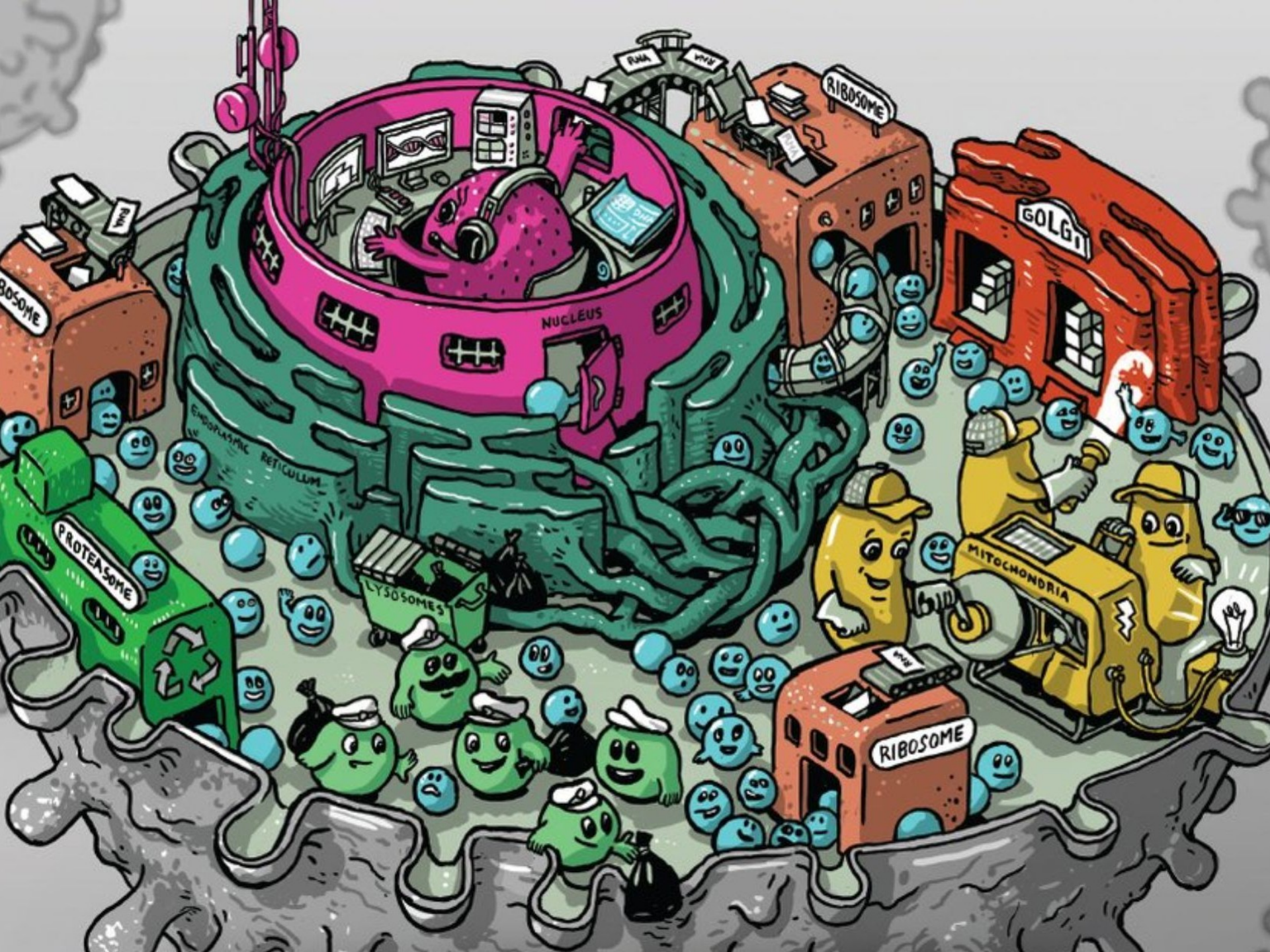
- reactions of cells to similar topographies on chemically different biomaterial surfaces are comparable

- major role of topography, modulated by the chemical surface coating

Health issues of nanomaterials

- **linked to multiple factors including chemical composition, size, shape, and surface chemistry**
- **entrance paths - inhalation through the respiratory tract, ingestion, injection into the blood stream, transportation via the skin**
- **historically - industrial manufacturing, gas exhaust from vehicles, coal, asbestos, man-made mineral fibers such as fiberglass, ambient particles in the atmosphere**
- **data available, but not yet conclusive**
- **who should be involved – scientists, industry, regulatory agencies, citizens interests groups, the public**

  – … not to repeat the fear of GMOs


RIBOSOME
GOLGI
NUCLEUS
ENDOPLASMIC RETICULUM
PROTEASOME
LYSOSOMES
MITOCHONDRIA
RIBOSOME