MUNI PHARM

Department of Natural Drugs

Supramolecular Pharmacy

11. Nanoparticles for drug delivery 2 (polymeric and inorganic NPs)

Ondřej Jurček

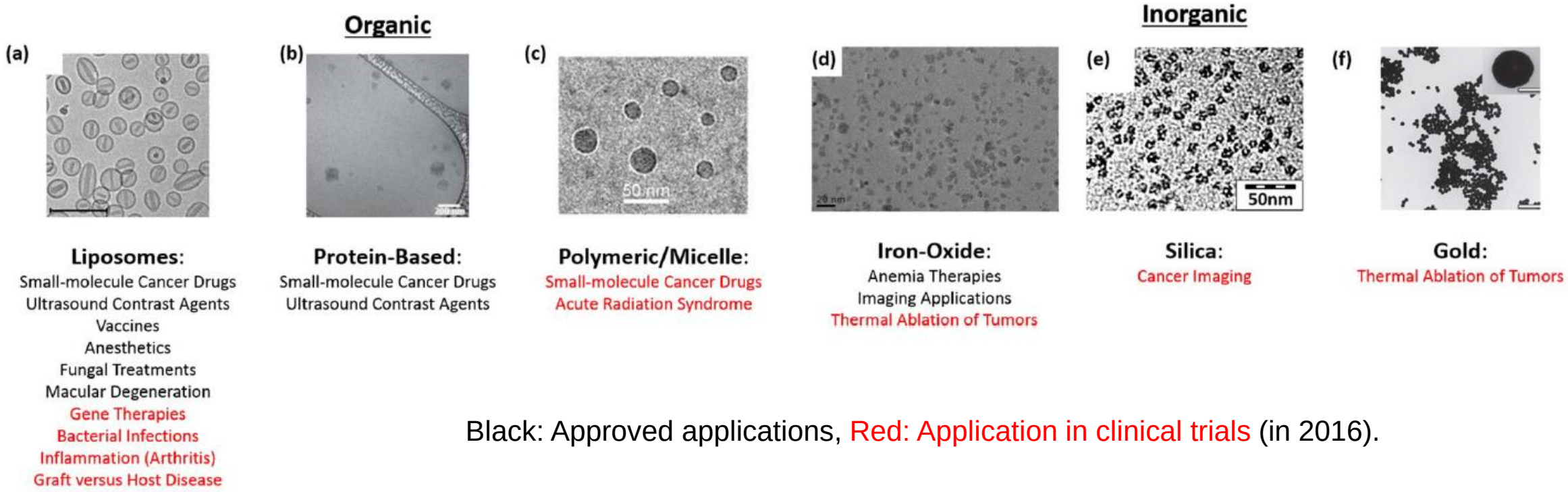
Nanoparticles (NPs) in drug delivery (nanocarriers)

- Oral, local, topical, and systemic nano-/microparticle delivery systems have been approved by FDA or EMA
- Intravenously administered NPs are the most studied preclinically and clinically
- Systemic delivery provides direct access to nearly all parts of the body
- ***Organic*** (including polymeric micelles and vesicles, liposomes, dendrimers, polymers, and hydrogels) and ***inorganic*** (including gold and mesoporous silica NPs)
- Porous NPs provide enhanced drug protection, controlled release, extended circulation, improved targeting to diseased tissues/cells
- Inorganic NPs provide the same and stimuli responsive functions due to surface plasmon resonance (thermal heating or imaging) or magnetic responsiveness (MRI, magnetic targeting)

Anselmo et al. *Bioengineering & Translational Medicine* 2016, 1, 10-29.

Clinically relevant NPs and their applications

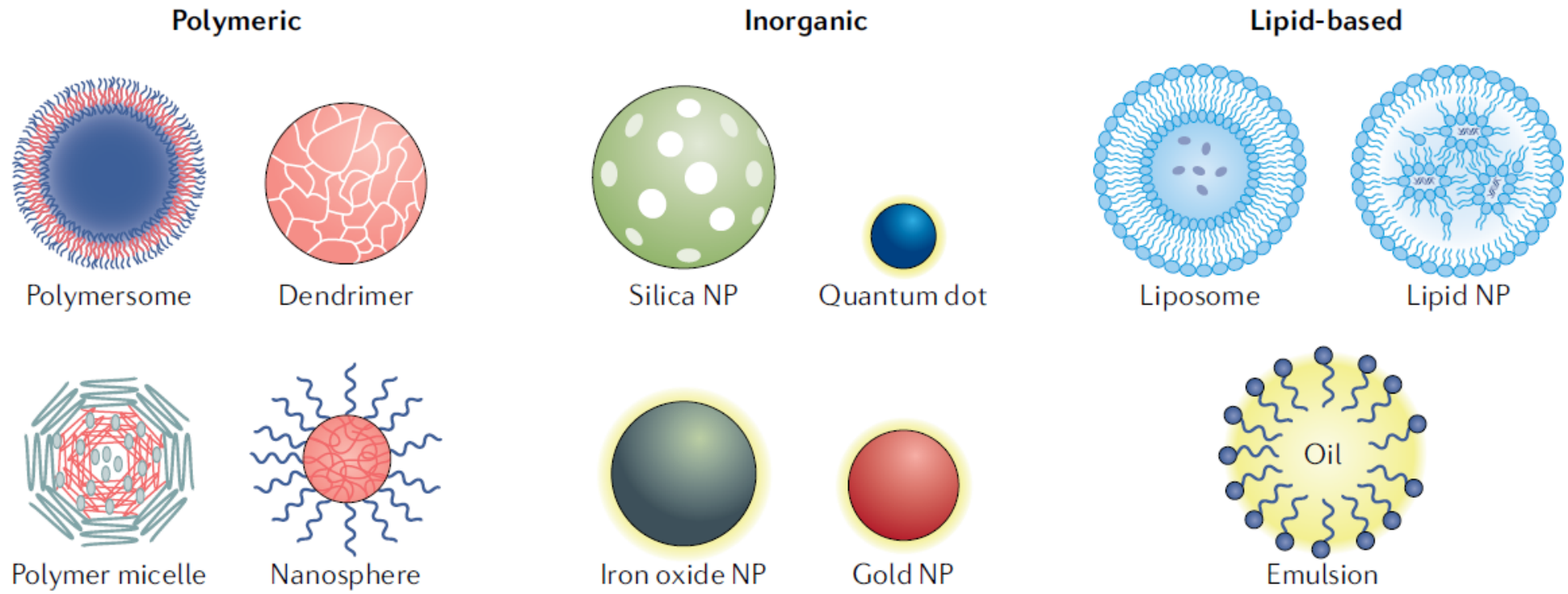
- Nanoparticles have been developed to overcome the limitations of free therapeutics and navigate biological barriers — systemic, microenvironmental and cellular — that are heterogeneous across patient populations and diseases
- Personalized delivery – precision medicine



Black: Approved applications, Red: Application in clinical trials (in 2016).

Anselmo et al. *Bioengineering & Translational Medicine* 2016, 1, 10-29.

Nanoparticles in pharmacy



- Precise control of particle characteristics
- Payload flexibility for hydrophilic and hydrophobic cargo
- Easy surface modification
- Possibility for aggregation and toxicity

- Unique electrical, magnetic and optical properties
- Variability in size, structure and geometry
- Well suited for theranostic applications
- Toxicity and solubility limitations

- Formulation simplicity with a range of physicochemical properties
- High bioavailability
- Payload flexibility
- Low encapsulation efficiency

Mitchell et al. *Nature Reviews: Drug Discovery* 2021, 20, 101–124.

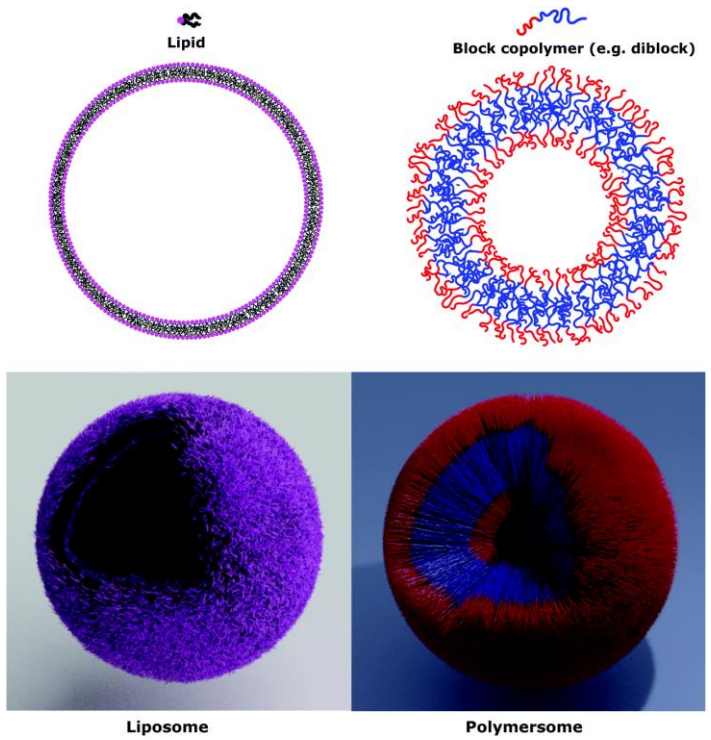
Polymeric nanoparticles

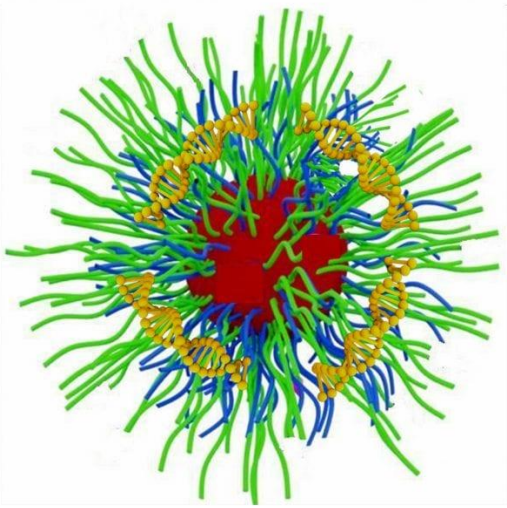
- Can be formed of biocompatible natural or synthetic polymers
- Therapeutics can be encapsulated within the NP core, entrapped in the polymer matrix, chemically conjugated to the polymer or bound to the NP surface
- Nanocapsules (cavities surrounded by a polymeric membrane or shell) and nanospheres (solid matrix systems)
- Modulating properties such as composition, stability, responsivity and surface charge, the loading efficacies and release kinetics of these therapeutics can be precisely controlled
- NPs are divided further into shapes such as polymersomes, micelles and dendrimers

Mitchell et al. *Nature Reviews Drug Discovery* 2021, 20, 101–124.

Polymeric nanoparticles

- ***Polymersomes*** are artificial vesicles, with membranes made using amphiphilic block copolymers. They are comparable to liposomes, but are reported to have improved stability and cargo-retention efficiency, making them effective vehicles for the delivery of therapeutics to the cytosol, *e.g.*, made of PEG, poly(dimethylsiloxane) (PDMS)
- ***Polymeric micelles*** are also typically responsive block copolymers, self-assemble to form nanospheres with a hydrophilic core and a hydrophobic coating: this serves to protect aqueous drug cargo and improve circulation times. Polymeric micelles can load various therapeutic types — from small molecules to proteins — and have been studied for the delivery of cancer therapeutics in clinical trials

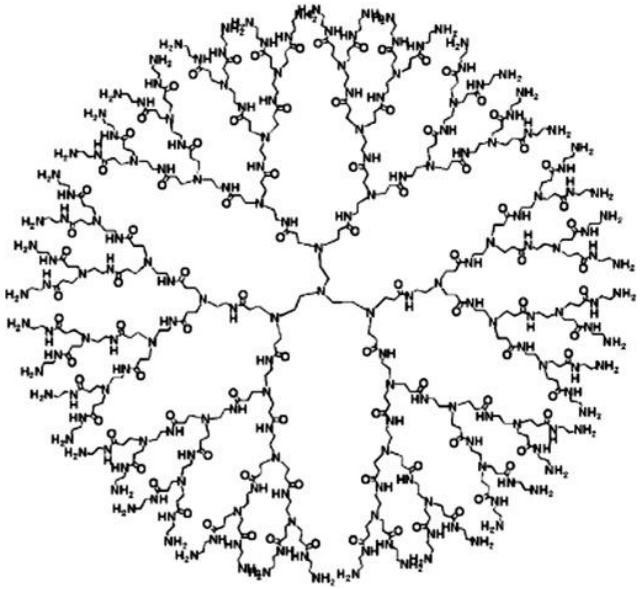


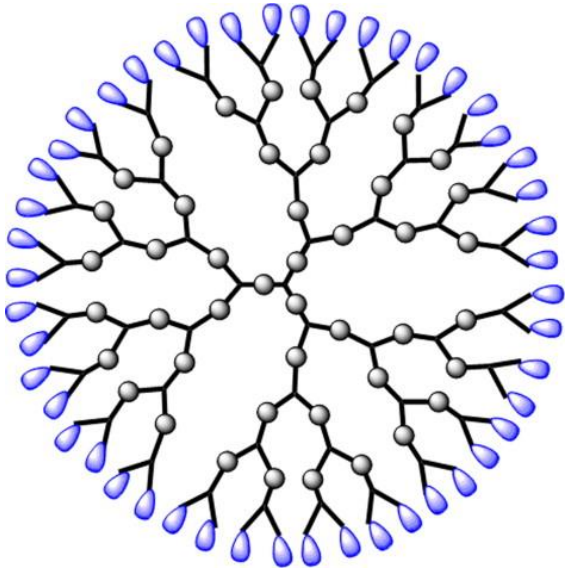


Mitchell et al. *Nature Reviews Drug Discovery* 2021, 20, 101–124.

Polymeric nanoparticles

- ***Dendrimers*** are hyperbranched polymers with complex three-dimensional architectures for which the mass, size, shape, and surface chemistry can be highly controlled. Active functional groups present on the exterior of dendrimers enable conjugation of biomolecules or contrast agents to the surface while drugs can be loaded in the interior. Dendrimers can hold many types of cargo but are the most commonly investigated for the delivery of nucleic acids and small molecules. Charged polymers such as poly(ethylenimine) (PEI) and poly(amidoamine) (PAMAM) are used.





Mitchell et al. *Nature Reviews Drug Discovery* 2021, 20, 101–124.

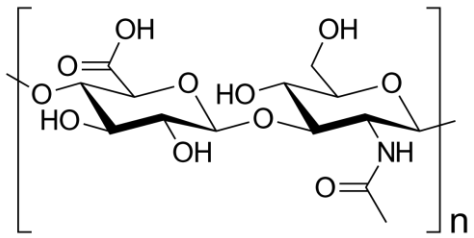
Polymeric nanoparticles

- Polymeric NPs have one important benefit, their scale-up and manufacturing are possible under Good Manufacturing Practices (GMP by WHO)
- Other benefits are significant stability of polymeric nanoparticles in biological fluids along with the wide availability of various polymers, the opportunity to functionalize their surfaces and to modulate polymer degradation and the leakage of the entrapped compound(s) as a function of specific stimuli
- Ideally the polymers must be biocompatible, biodegradable, and possess specific mechanical and physicochemical properties
- The first polymers used to develop polymeric nanoparticles (PNs) were non-biodegradable polymers, such as poly(methylmethacrylate) (PMMA), polyacrylamide, polystyrene, and polyacrylates. The nanosystems made up of these materials exhibited a rapid and efficient clearance, also, chronic toxicity and inflammatory reactions were observed
- Degradation rate of biodegradable polymeric nanoparticles can be influenced by several parameters, including their physicochemical properties (size, structure, molecular weight) and external factors, such as pH and temperature

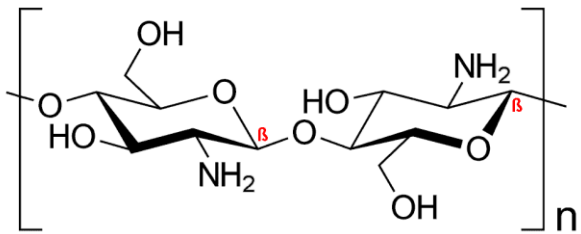
Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Polymers for nanoparticles

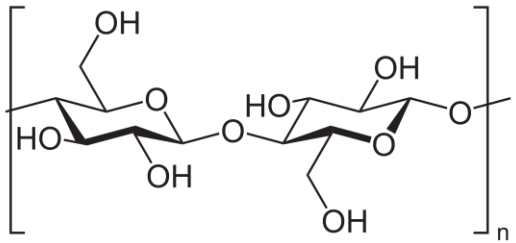
- Synthetic and non-synthetic biodegradable polymers
- ***Synthetic biopolymers***: include synthetic polymers such as poly(D,L-lactide) (PLA), poly(D,L-glycolide) (PLG), co-polymer poly(lactide-co-glycolide) (PLGA), polyalkylcyanoacrylates, poly-Ɛ-caprolactone, polyhydroxyalkanoates (PHAs), and poly(alkyl cyanoacrylates) (PACA) (some approved by FDA and EMA)
- ***Non-synthetic natural biopolymers***:
  - Animals – albumin nanoparticles (Abraxane®), gelatin, hyaluronic acid
  - Plants – cellulose, starch, soy protein, zein
  - Marine organisms – chitosan (cationic), alginate, carrageenan



Hyaluronic acid



Chitosan



Cellulose

Albumin

Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Preparation of polymeric NPs

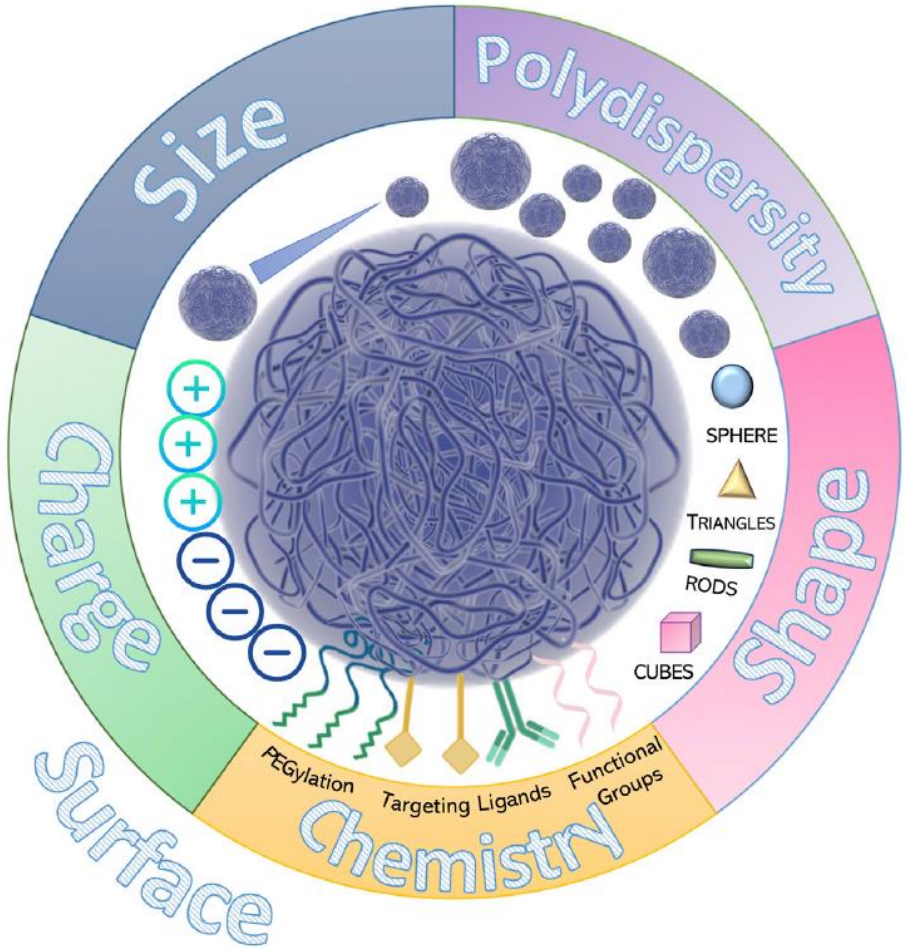
- The most important: ***emulsification and solvent evaporation*** (small-moderate scale manufacturing: Polymer in organic solvent into water with surfactants and stabilizers – emulsification – evaporation of organic solvent (spray-drying)
- ***Nanoprecipitation***: water miscible organic solvent with polymer (drug) is slowly dropped into aqueous solution
- ***Supercritical anti-solvent method***: polymeric solutions sprayed into CO_2 under high pressure
- ***Salting-out***: electrolytes such as magnesium chloride, calcium chloride and magnesium acetate or saccharides (non-electrolyte) added to a polymeric solution

- The length of polymer plays an important role (effects also degradation), stabilizers..
- Prevent aggregation – electrostatic and steric stabilization (surfactants)
  - Surfactants: (i) anionic (negative charge); (ii) cationic (positive charge); (iii) zwitterionic or amphoteric (charge depends on the pH of the medium), and; (iv) non-ionic (no charge)
- Vitamin E TPGS (vitamin E D-α-tocopheryl-polyethylene glycol 1000 succinate) enhances drug encapsulation, cellular uptake, therapeutic efficacy, and oral bioavailability of nanocarriers

Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Physico-chemical properties of polymeric NPs

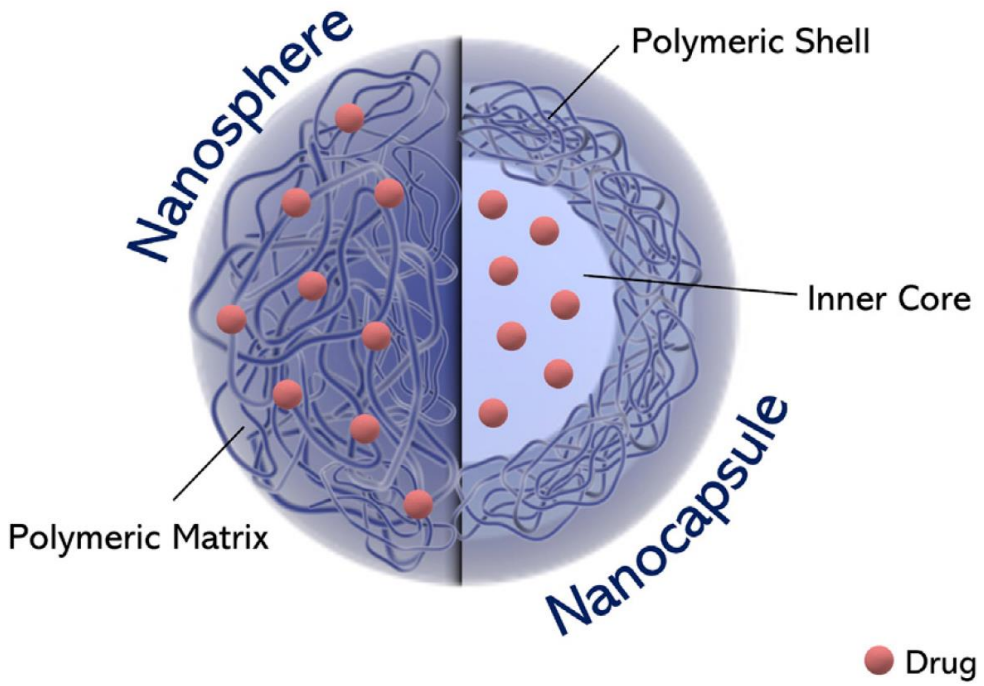
- Size, shape, stability, drug-release profiles and surface characteristics
- The European Nanomedicine Characterization Laboratory (EUNCL) and the US National Cancer Institute Nanotechnology Characterization Laboratory (NCI-NCL) have developed multiple standard operating procedures for nanomaterial assessment, establishing mean size and polydispersity index as the critical quality attributes (~100 nm)
- Interaction with a biomaterial could favor the formation of aggregates/particles of different mean sizes, leading to significant differences in cell uptake and distribution, toxic effects, and fate within the cell



Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Drug

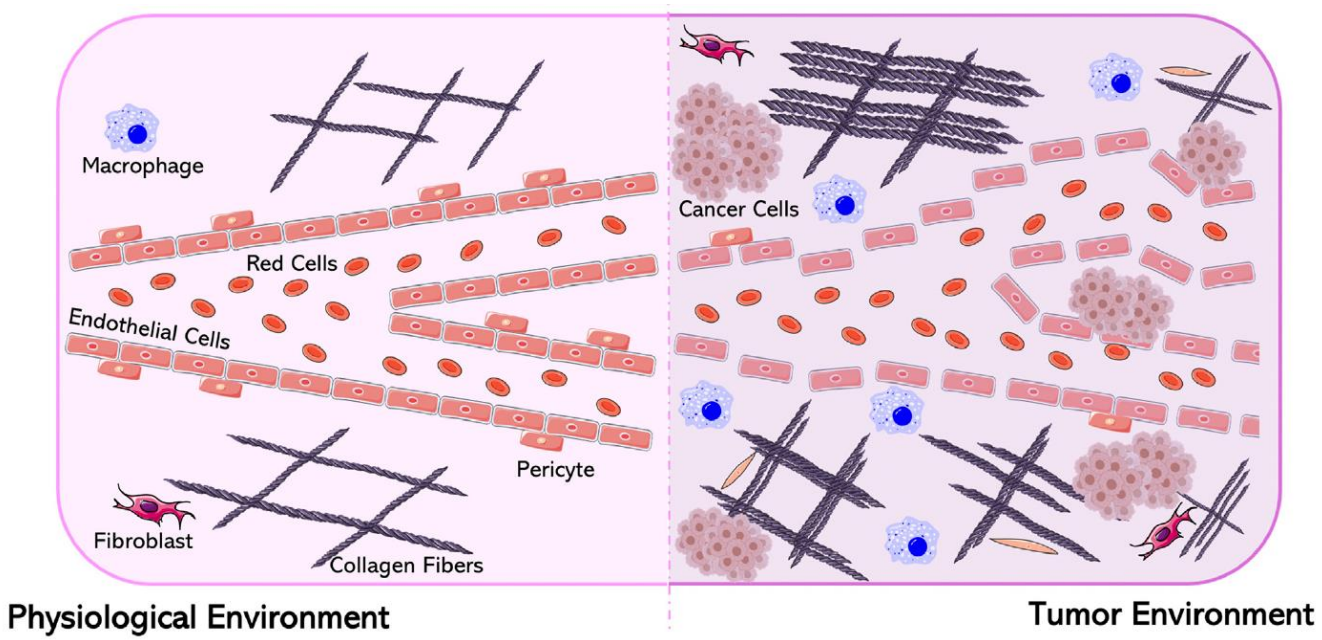
- Encapsulating hydrophilic and hydrophobic molecules, such as salts, proteins, and high-molecular-weight DNA or antisense nucleic acids
- Drugs encapsulated in PNPs are released by means of diffusion through the polymeric network, erosion of the matrix material, hydrostatic swelling, or by a combination of these mechanisms



Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Tumor microenvironment

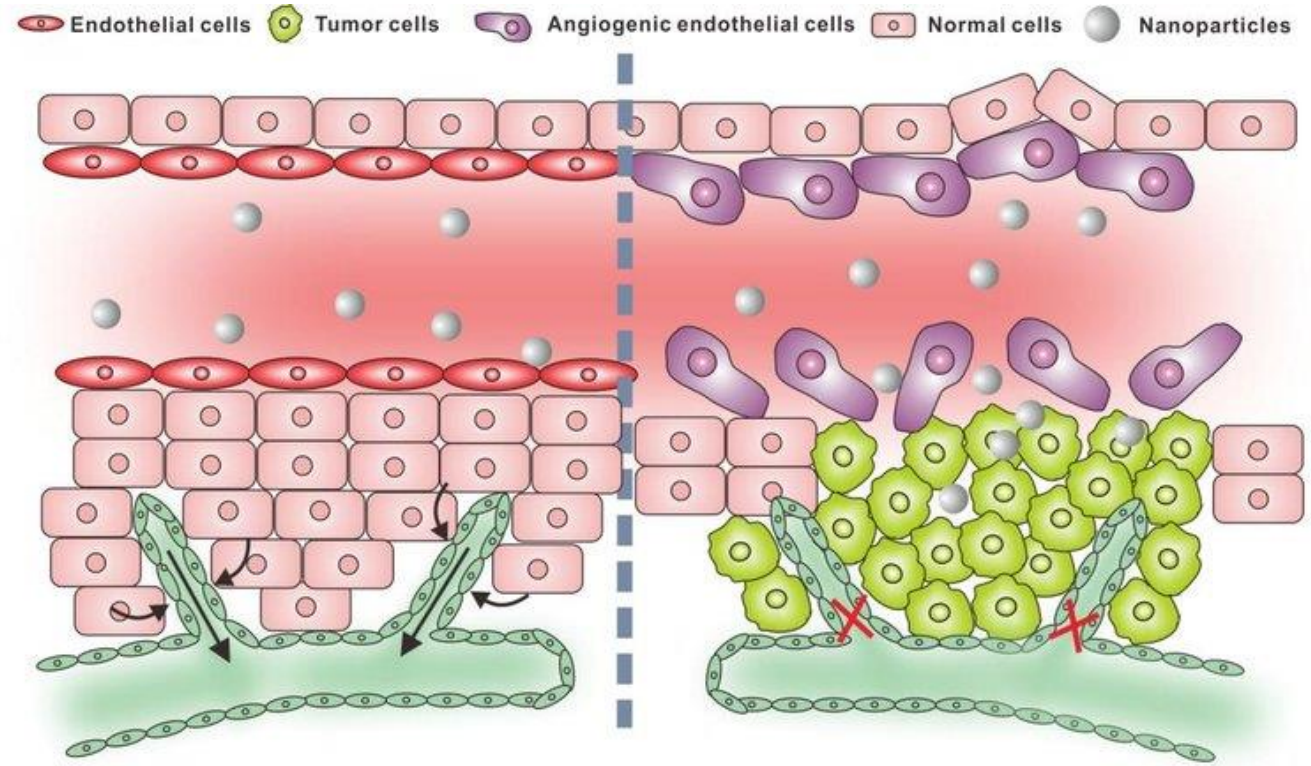
- Large differences between tumor and healthy tissues, e.g., vascular abnormalities, oxygenation and perfusion levels, pH, and metabolic status
- Solid tumors are characterized by a heterogeneous population of neoplastic cells supplied by an irregular and discontinuous endothelium with large gaps between the endothelial cells, and abnormally thick or thin basement membranes where pericites are loosely attached to endothelial cells
- The abnormal and disorganized tumor vasculature results in inefficient blood flow inside the tumor mass, hypoxia, and low extracellular pH



Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Enhanced permeability and retention (EPR) effect

- high–molecular weight nontargeted drugs and prodrugs accumulate in tissues that offer increased vascular permeability, such as in sites of inflammation or cancer



Xie et al. *Curr. Cancer Drug Target* **2019**, *19*(4), 257.

Tumor microenvironment

- Hypoxia plays a crucial role in tumor growth and metastasis through the induction of molecular signaling which is responsible for genetic instability, inflammation, immunosuppression, epithelial–mesenchymal transition and altered metabolism
- It also confers resistance against several kinds of treatment, such as radiation, chemo-, photodynamic and immunotherapies, which require oxygen for efficacy
- Oxygen can diffuse for maximum 150 μm beyond the capillary wall, which implies that when a tumor reaches a certain size (~2mm^3), a state of cellular hypoxia begins
- Angiogenesis is a cellular mechanism which is upregulated in tumoral microenvironments and creates new blood vessels to further assist tumor growth by supplying oxygen and nutrients
- Hypoxia also results in metabolic acidosis caused by increased glycolysis (the Warburg effect); this lowers the extracellular pH to 6.0–7.0
- The elevated breakdown of glucose produces large amounts of lactic acid and significant amounts of free protons (H^+) which are pumped into the extracellular milieu

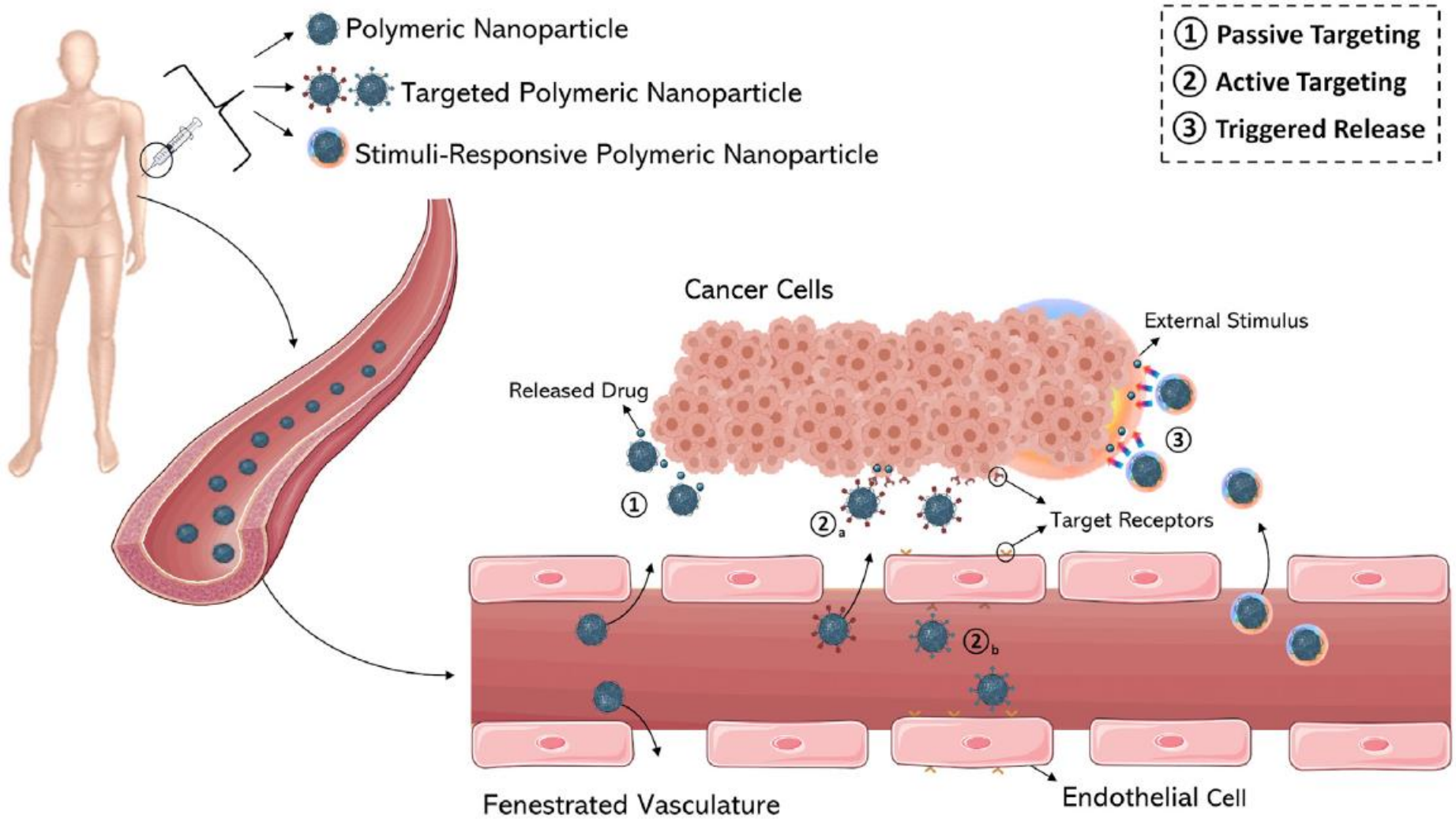
Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Tumor microenvironment

- The resulting pH gradients between intra- and extracellular compartments within the tumor tissue, as well as between the tumor mass and the general host tissue, are potential sources of variable and often inefficient partitioning and distribution of drugs
- Exposure to chemotherapy may favor the selection of tumor-cell clones with acidic organelles, which are able to entrap the drugs, and if these organelles are part of the secretory pathway, then the drug will be transported out of the cell through exocytosis. All these factors in the tumor microenvironment contribute to multidrug resistance (MDR) phenomena

Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Various drug targeting approaches



Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Nanoparticle passive targeting and PEGylation

- ***Passive targeting:*** Convection (large molecules travel through large pores) or passive diffusion (concentration gradient, small molecules) – enhanced permeability and retention effect (EPR) – 100-800 nm
- Polymeric nanoparticles, micelles, liposomes, and dendrimers of 80–150 nm are retained in the solid tumor tissue, but smaller particles (<20–30 nm) can easily diffuse into other compartments

- Total amount of protein adsorbed onto the surfaces of the nanoparticles significantly decreased when the M_W of PEG was a maximum of 5 kDa
- AntiPEG-antibodies - at the end of the last century, only 0.2% of the population had them, while since 2012 a dramatic increase in the number (up to 25%) has been observed - “accelerated blood clearance” (ABC)

- ***Active targeting*** moieties: antibodies, glucose, transferrin, folate, transporters and integrin ligands

Gagliardi et al. *Frontiers in Pharmacology* 2021, 12, 601626.

Polymeric nanoparticles

- Overall, polymeric NPs are ideal candidates for drug delivery because they are biodegradable, water soluble, biocompatible, biomimetic and stable during storage. Their surfaces can be easily modified for additional targeting — allowing them to deliver drugs, proteins and genetic material to targeted tissues, which makes them useful in cancer medicine, gene therapy and diagnostics.

- However, disadvantages of polymeric NPs include an increased risk of particle aggregation and toxicity. Only a small number of polymeric nanomedicines are currently FDA approved and used in the clinic, but still polymeric nanocarriers are undergoing testing in numerous clinical trials

Mitchell et al. *Nature Reviews Drug Discovery* 2021, 20, 101–124.

Polymer-based nanoparticles approved by FDA

Clinical products	Formulation	Indication	Company	Year
		Polymer-based nanoparticles		
Renagel	Poly(allylamine hydrochloride)	Chronic kidney disease	Sanofi	2000
Eligard	Leuprolide acetate and polymer PLGA (poly (DL-lactide-co-glycolide))	Prostate cancer	Tolmar	2002
Estrasorb	Micellar estradiol	Menopausal therapy	Novavax	2003
Cimzia/certolizumab pegol	PEGylated antibody fragment (certolizumab)	Crohn's disease Rheumatoid/psoriatic arthritis Ankylosing spondylitis	UCB	2008-2013
Genexol-PM	mPEG-PLA micelle loaded with paclitaxel	Metastatic breast cancer	Samyang Corporation	2007 South Korea
Adynovate	Polymer-protein conjugate (PEGylated factor VIII)	Hemophilia	Baxalta	2015

Lombardo et al. *Journal of Nanomaterials* 2019, Article ID 3702518.

Inorganic nanoparticles

- Inorganic materials such as gold, iron, and silica have been used to synthesize nanostructured materials for various drug delivery and imaging applications
- Variety of sizes, structures and geometries, *e.g.*, gold NPs can form nanospheres, nanorods, nanostars, nanoshells, and nanocages
- Inorganic NPs have unique physical, electrical, magnetic and optical properties, due to the properties of the base material itself
- For example, AuNPs possess free electrons at their surface that continually oscillate at a frequency dependent on their size and shape (surface plasmon resonance), giving them photothermal properties
- AuNPs are also easily functionalized, granting them additional properties and delivery capabilities

Mitchell et al. *Nature Reviews Drug Discovery* 2021, 20, 101–124.

Inorganic nanoparticles

- Iron oxide is another commonly researched material for inorganic NP synthesis, and iron oxide NPs make up the majority of FDA- approved inorganic nanomedicines

Inorganic			
INFeD	Allergan	Iron-deficient anaemia	1992
DexFerrum	American Regent	Iron-deficient anaemia	1996
Ferrlecit	Sanofi	Iron deficiency in chronic kidney disease	1999
Venofer	American Regent	Iron deficiency in chronic kidney disease	2000
Feraheme	AMAG	Iron deficiency in chronic kidney disease	2009
Injectafer	American Regent	Iron-deficient anaemia	2013

- Magnetic iron oxide NPs — composed of magnetite (Fe_3O_4) or maghemite (Fe_2O_3) — possess superparamagnetic properties at certain sizes and have shown success as contrast agents, drug delivery vehicles, and thermal-based therapeutics

Mitchell et al. *Nature Reviews Drug Discovery* 2021, 20, 101–124.

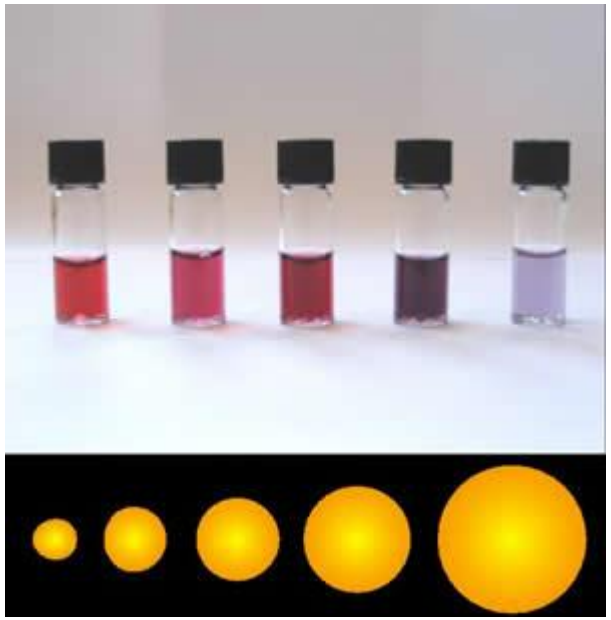
Inorganic nanoparticles

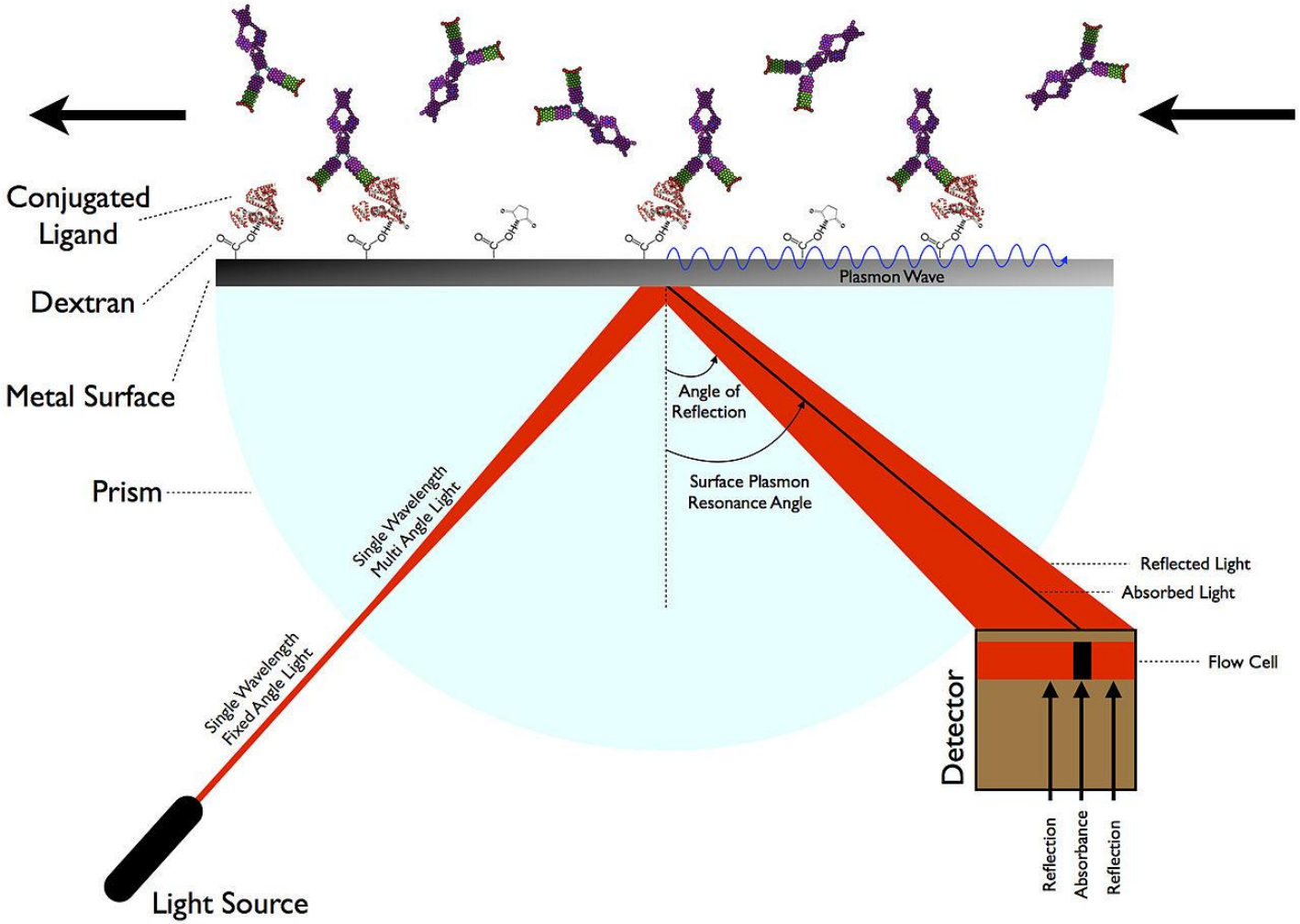
- Inorganic NPs include calcium phosphate and mesoporous silica NPs, which have both been used successfully for gene and drug delivery
- Quantum dots — typically made of semiconducting materials such as silicon — are unique NPs used primarily in *in vitro* imaging applications, but they show promise for *in vivo* diagnostics
- Due to their magnetic, radioactive or plasmonic properties, inorganic NPs are uniquely qualified for applications such as diagnostics, imaging and photothermal therapies. Most have good biocompatibility and stability, and fill niche applications that require properties unattainable by organic materials. However, they are limited in their clinical application by low solubility and toxicity concerns, especially in formulations using heavy metals

Mitchell et al. *Nature Reviews Drug Discovery* 2021, 20, 101–124.

Gold nanoparticles

- Noble metal NPs (Au, Pt, Ag) have ***surface plasmon resonance*** (***SPR***, nano-sized photon confinement)

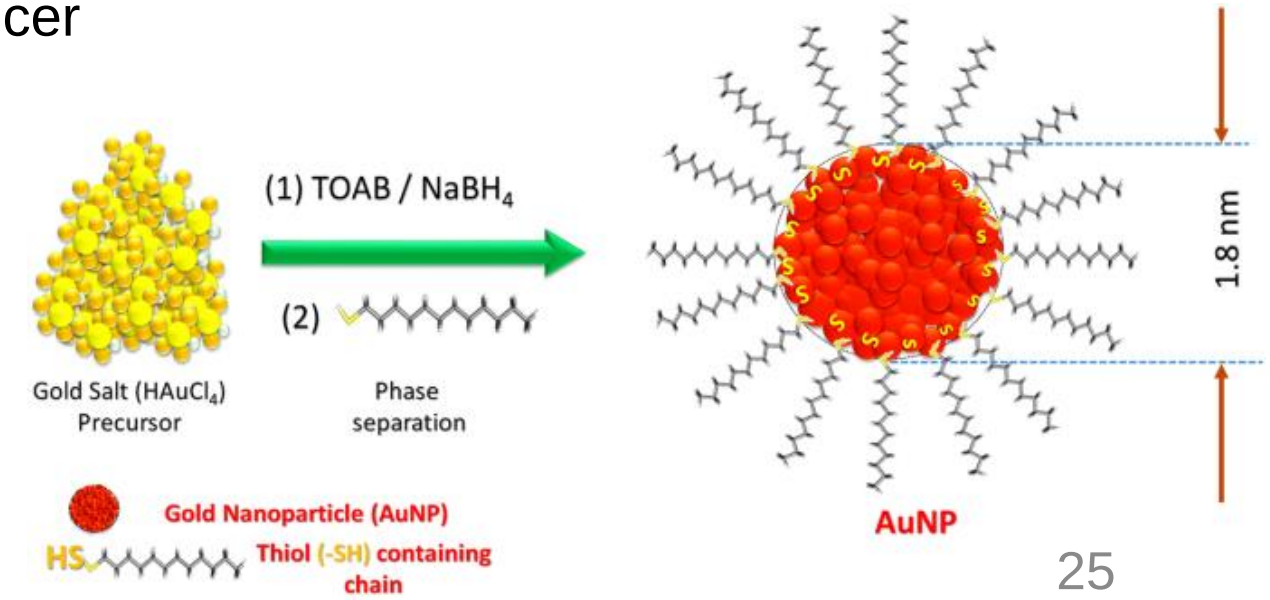




Wikipedia

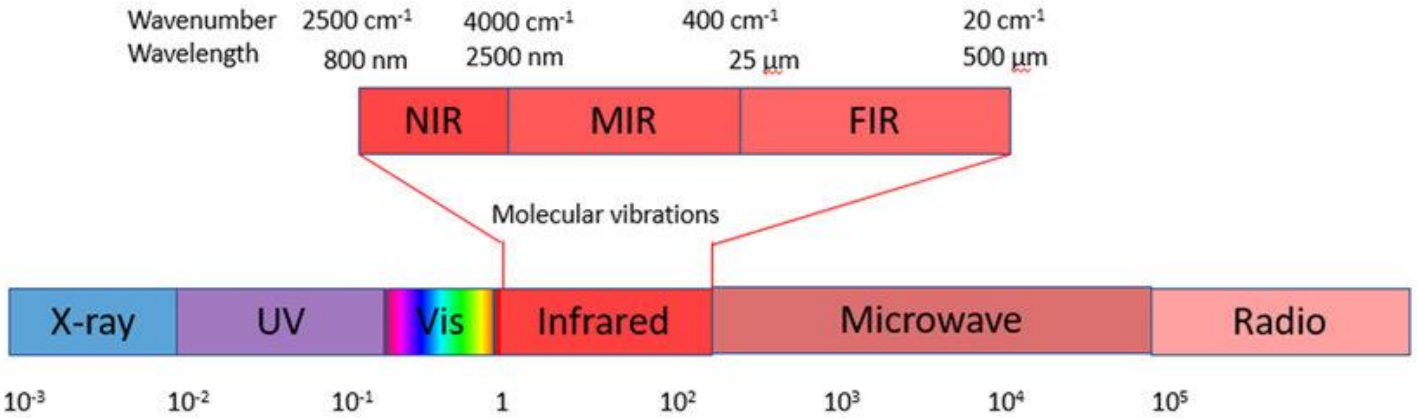
Gold nanoparticles as drugs

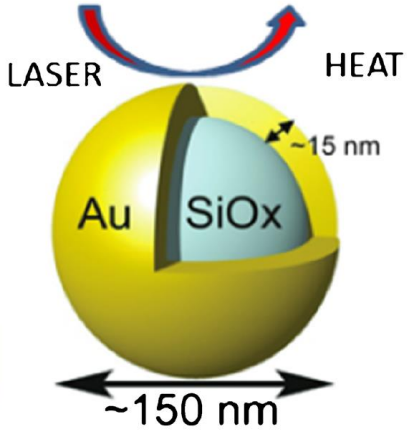
- Imaging – light scattering × tumor ablation – photothermal properties (light absorption)
- Properties dependent on size and shape of NPs
- Traditionally, loading of AuNPs with a variety of therapeutic molecules through surface modification (affinity of AuNPs to thiols)
- Limited toxicity to cells
- The total extinction of light at the SPR is made up of both absorption and scattering. For the smaller axial diameter nanorods (~10 nm), absorption dominates, whereas for the larger axial diameter nanorods (>35 nm) scattering can dominate.
- Aurimune, a tumor necrosis factor-bound AuNP, has completed key clinical phase 1 trials (no drug approved yet) for treatment of cancer
- AuroLase® is being developed by Nanospectra, are silica-gold nanoshells coated with (poly)ethylene glycol (PEG) designed to thermally ablate solid tumors following stimulation with a near-infrared energy source

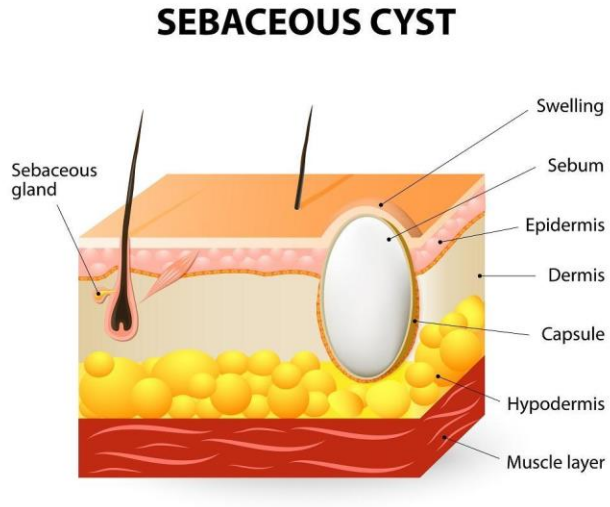


Anselmo et al. *Am. Assoc. Pharm. Sci.* 2015, 17(*5*), 1041.

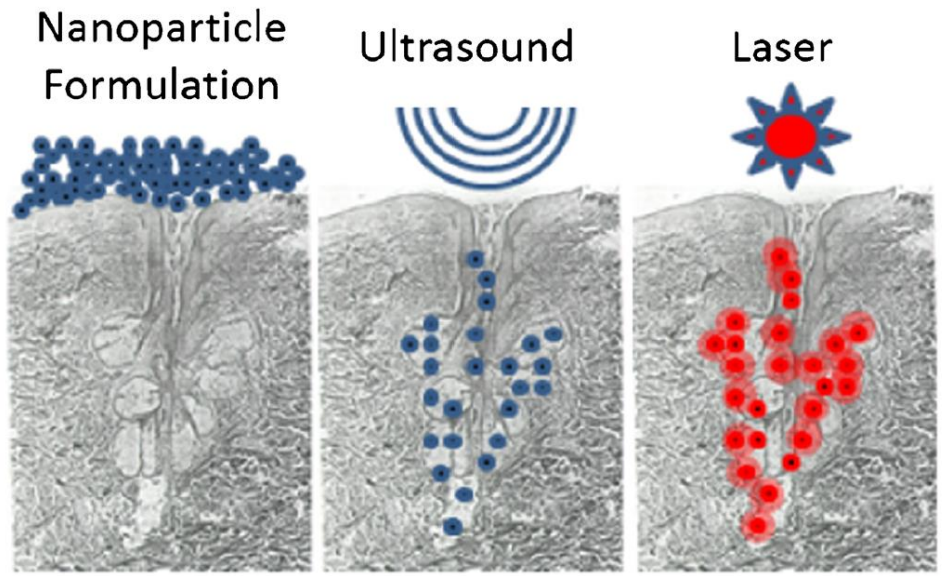
Gold nanoparticles as drugs







- AuNPs capable of treating acne (Sebashells) are being developed by Sebacia Inc
  - ~150 nm silica-gold nanoshells, coated with PEG
  - treat acne by disrupting overactive sebaceous glands in the skin



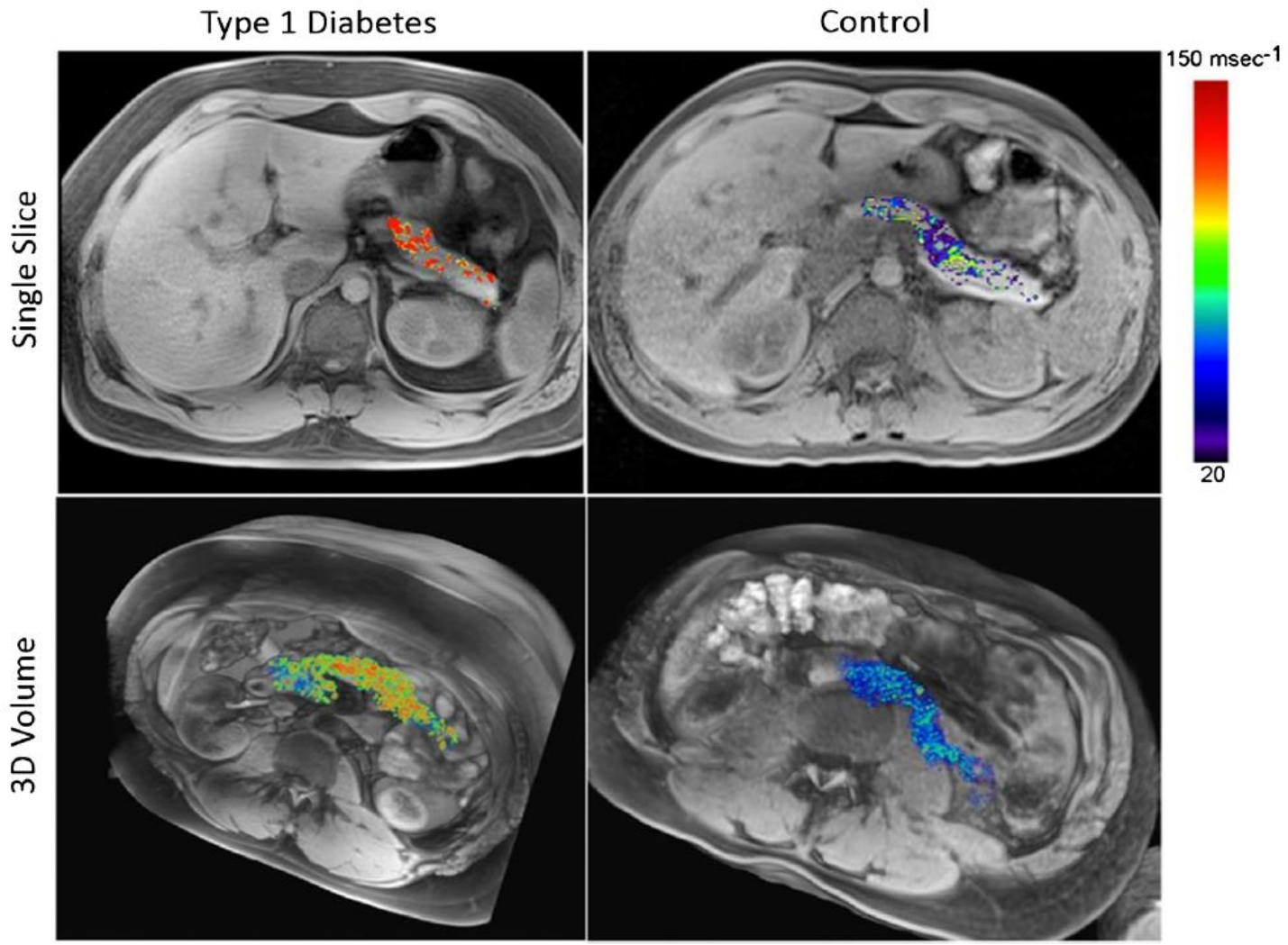
Anselmo et al. *Am. Assoc. Pharm. Sci.* 2015, 17(*5*), 1041.

Iron oxide nanoparticles

- Iron oxide nanoparticles (IONPs), either superparamagnetic iron oxides (SPIO) or ultrasmall superparamagnetic iron oxides (USPIO)
- Properties: *innate magnetic responsiveness* facilitating targeting, imaging, localized heating (hyperthermia, tumor ablation); *biocompatibility and biodegradability*
- IONPS are important contrast agents for noninvasive diagnostic imaging using magnetic resonance imaging (MRI)
- These are the most clinically studied inorganic NPs, approved by FDA for imaging of different pathologies and for treatment of iron deficiency (anemia)
- Ferumoxytol are USPIO nanoparticles (17-31 nm) coated with polyglucose sorbitol carboxymethylether – approved for treatment of anemia of patients with chronic kidney disease or studied as imaging agent of a variety of diseases and conditions, ranging from: multiple sclerosis to numerous cancers (*e.g.*, prostate, bladder, breast, lung, ovarian, to name a few) to heart conditions to type 1 diabetes
- Generally, IONPs could be used to: (i) enhance targeting to diseased tissues *via* magnetic field, (ii) deliver loaded drugs to this target site, and (iii) be activated externally to destroy surrounding tissue, effectively providing a targeted combination therapy.

Anselmo et al. *Am. Assoc. Pharm. Sci.* 2015, 17(*5*), 1041.

Ferumoxytol imaging



Anselmo et al. *Am. Assoc. Pharm. Sci.* 2015, 17(*5*), 1041.

Iron-based nanoparticles approved by FDA

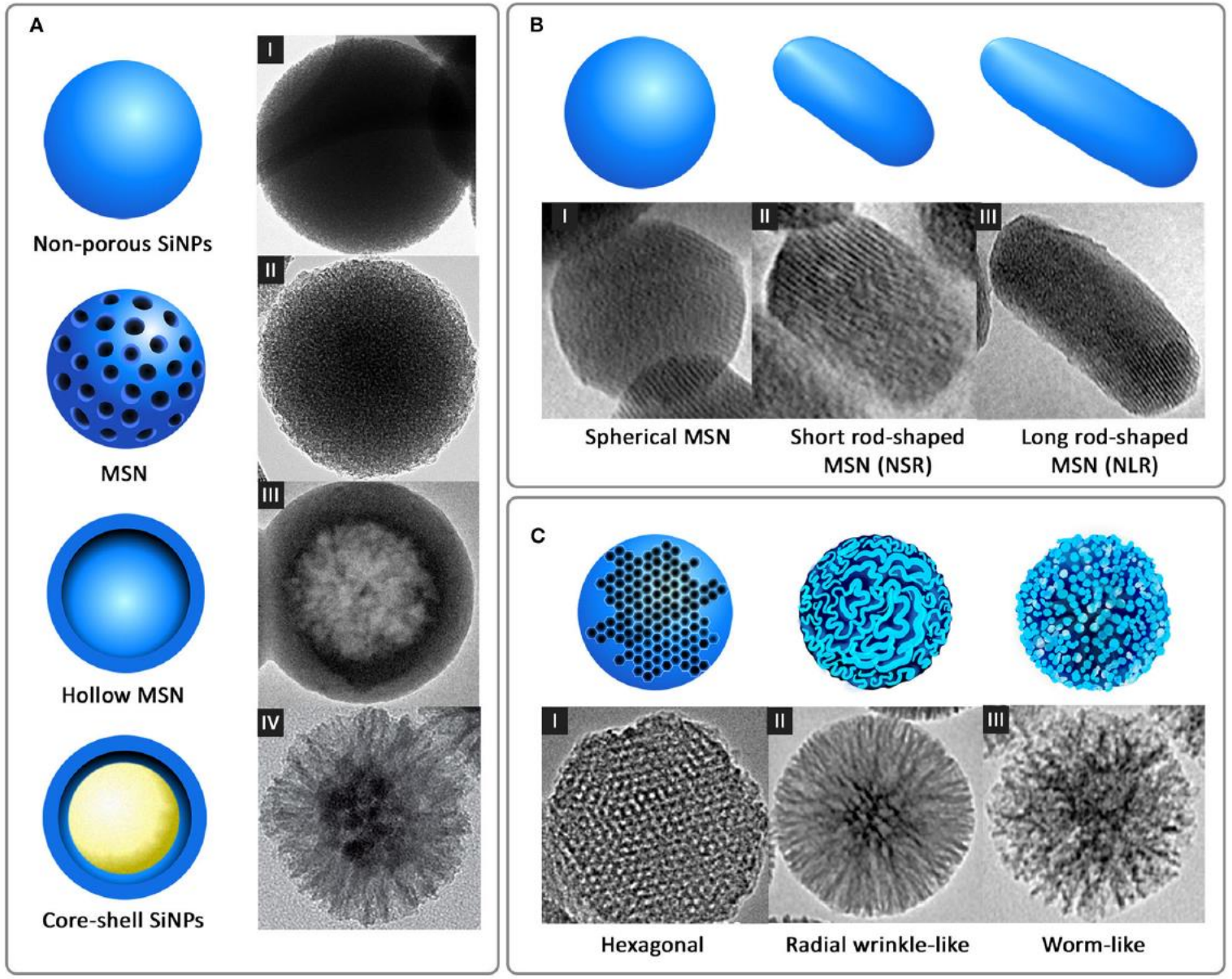
Name	Material/functionality	Application/indication	Approval date
Feridex I.V.®; Endorem®	Iron oxide nanoparticles (coated with dextran) Magnetic-field responsive for MRI imaging	Imaging of liver lesions	FDA approved (1996). Discontinued (2008)
Resovist®; Cliavist	Iron oxide nanoparticles (coated with carboxydextran) Magnetic-field responsive for MRI imaging	Imaging of liver lesions	EMA approved (2001). Discontinued (2009)
Gastromark™; Lumirem®	Iron oxide nanoparticles (coated with silicone) Magnetic-field responsive nanoparticles for MRI imaging	Enhance bowel imaging (oral administration)	FDA approved (1996). Discontinued (2012)
Ferumoxtran-10; Combidex®; Sinerem®	Iron oxide nanoparticles (coated with dextram) Magnetic-field responsive for MRI imaging	Lymph node metastases imaging	Approved in some European countries. Application withdrawn from EMA (Sinerem 2007). Application withdrawn from FDA (2005)
Feraheme®; Rienso®; Ferumoxytol	Iron oxide nanoparticles (coated with polyglucose sorbitol carboxymethylether)	Treatment of iron deficiency in adults with chronic kidney disease	FDA approved (2009)

Anselmo et al. *Am. Assoc. Pharm. Sci.* 2015, 17(*5*), 1041.

Silica-based NPs

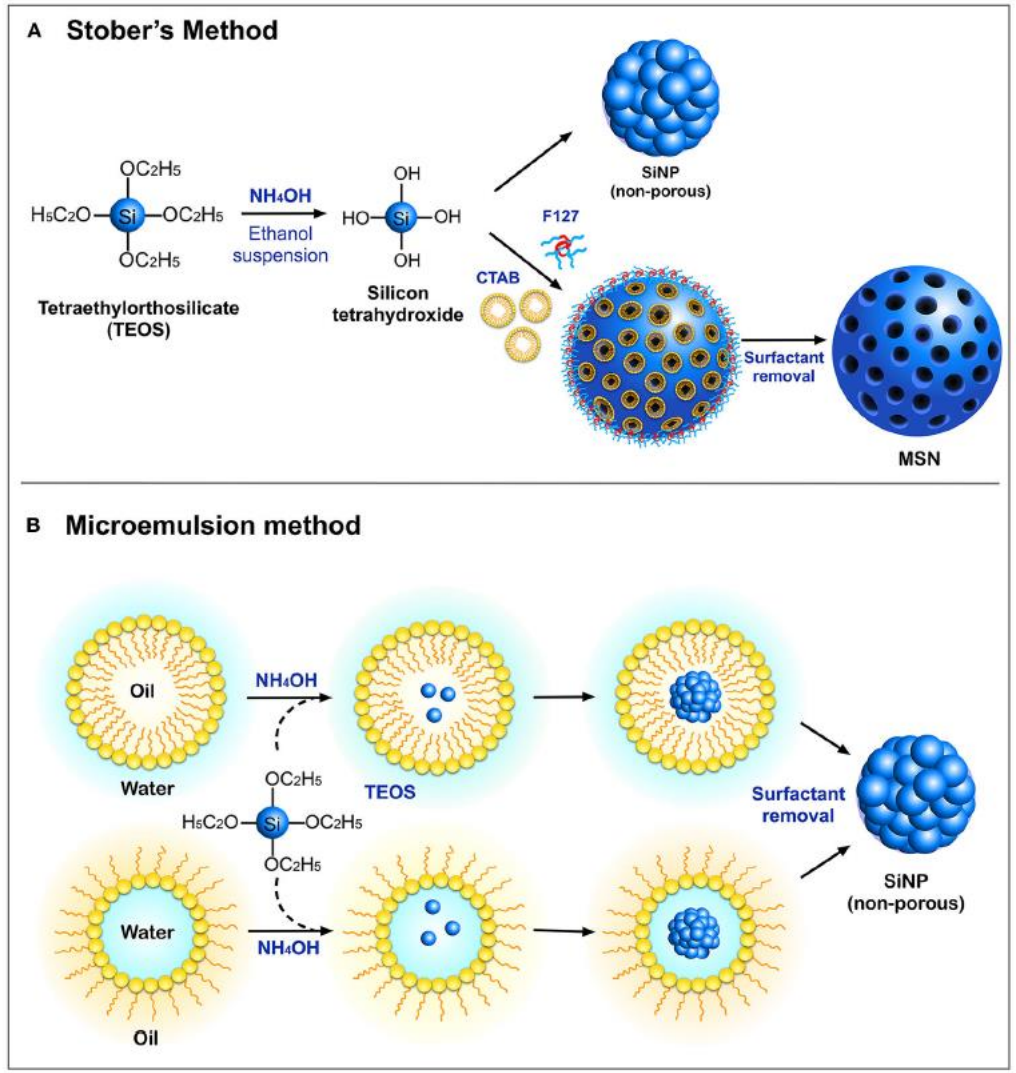
- Or silicon dioxide (SiO_2) are tunable materials where we can control the size, shape, and porosity + do surface modifications (conjugation of various stealth or targeting ligands to influence circulation, targeting, drug loading and release, and internalization by cells of intravenously administered NPs
- Silica NPs have limited toxicity and favorable biodegradability

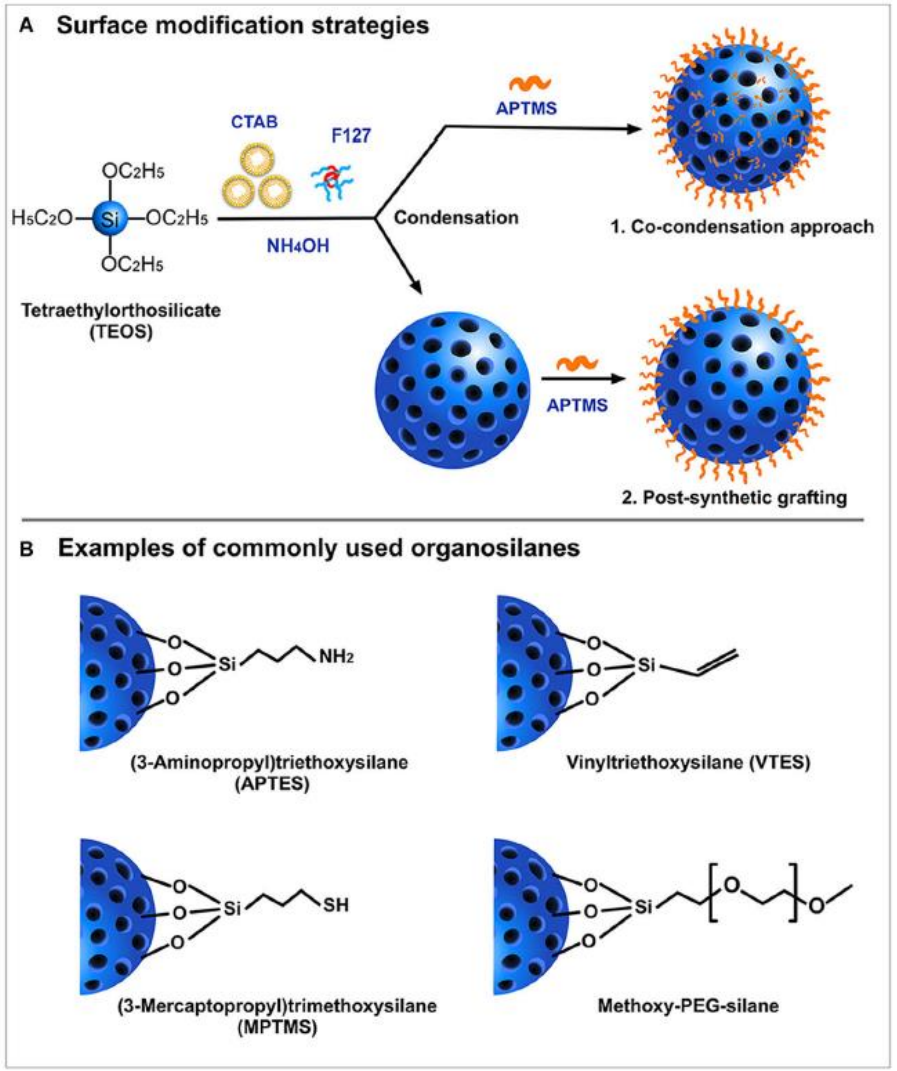
- Synthesis, e.g., by Stober‘s or microemulsion method



Selvarajan et al. *Front. Chem.* 2020, 8, Article 602

Synthesis and surface modification of silica NPs





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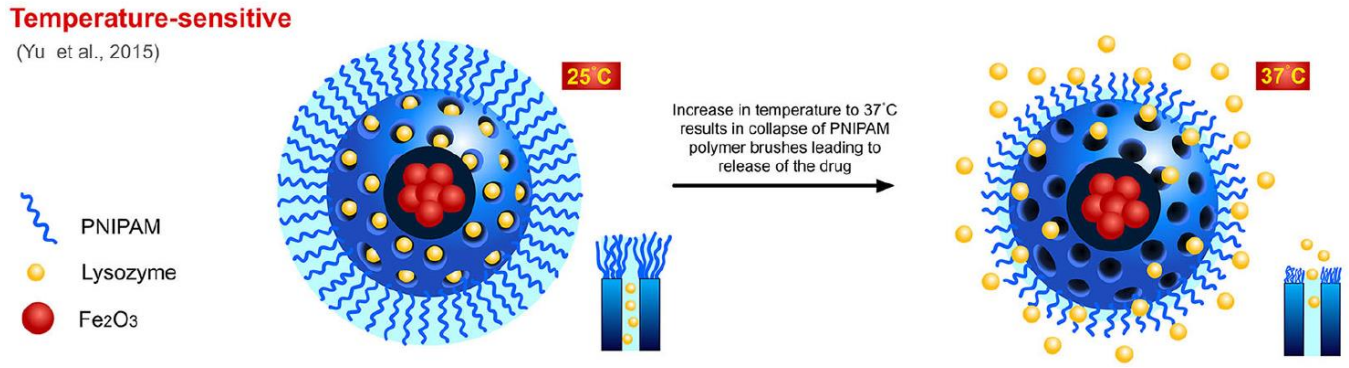
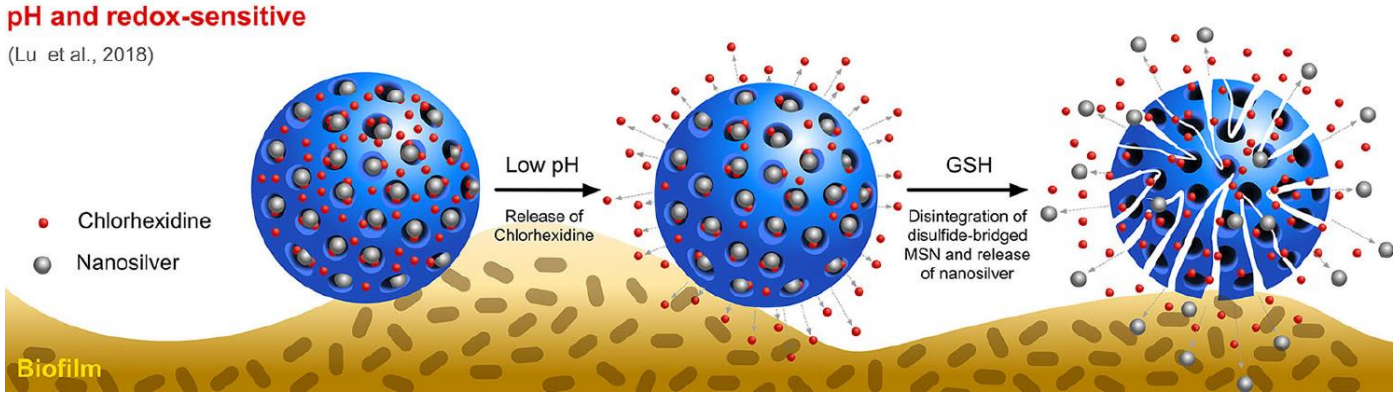
Silica-based NPs (SNPs) in drug delivery

- incorporation of hydrophilic or hydrophobic drugs
- diagnostic applications, including imaging, gene transfection vectors for cellular uptake, and stimuli-responsive delivery (many SNPs are used in combination with other inorganic materials (*e.g.*, gold as in AuroLase®))
- One of the main challenges facing SNPs is determining their long-term *in vivo* fate if they are not cleared by the kidneys





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In the next class…

Photomedicine

Thank you for your attention!