Gene Therapy:
Non viral Gene Therapy

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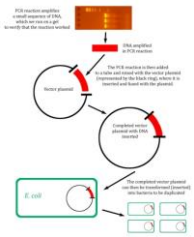
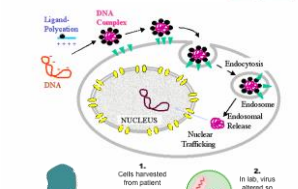
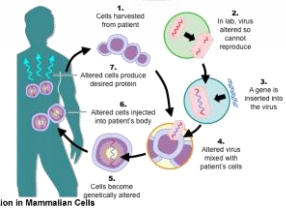
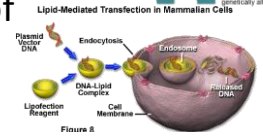
Gene Transfer

- **Transformation:** introduction of genetic materials into bacteria
- **Transfection:** introduction of genetic materials into eukaryotic cells (e.g. fungi, plant, or animal cells)
- **Transduction:** introduction of genetic materials ***using*** viruses
- **Lipofection:** introduction of genetic materials ***using*** liposomes

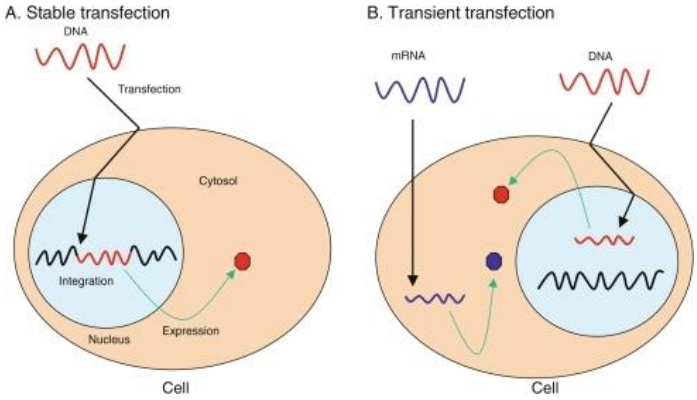








Stable vs. Transient Gene Transfer



- **Stable Gene Transfer:** achieved by plasmid integration in the host genome or episomal replication of the transferred plasmid.
- **Transient Gene Transfer:** the foreign DNA is usually not integrated into the nuclear genome and will be degraded or diluted through mitosis

Design plasmid

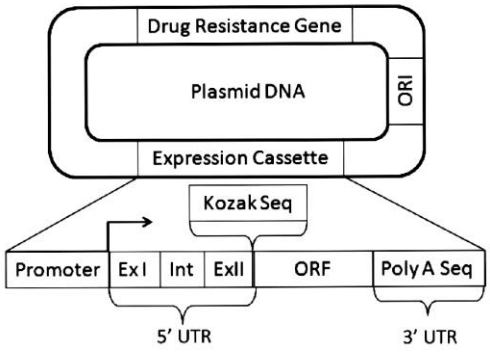


Fig. 1.1 Diagram of Plasmid DNA. Diagram shows feature of plasmid DNA. Abbeviations: ORI-Origin of Replication; ExI and ExII-Exon I and Exon II; Int-Intron; Poly A Seq-Poly Adenylation Sequence; Arrow-Transcription Initiation Sequence.

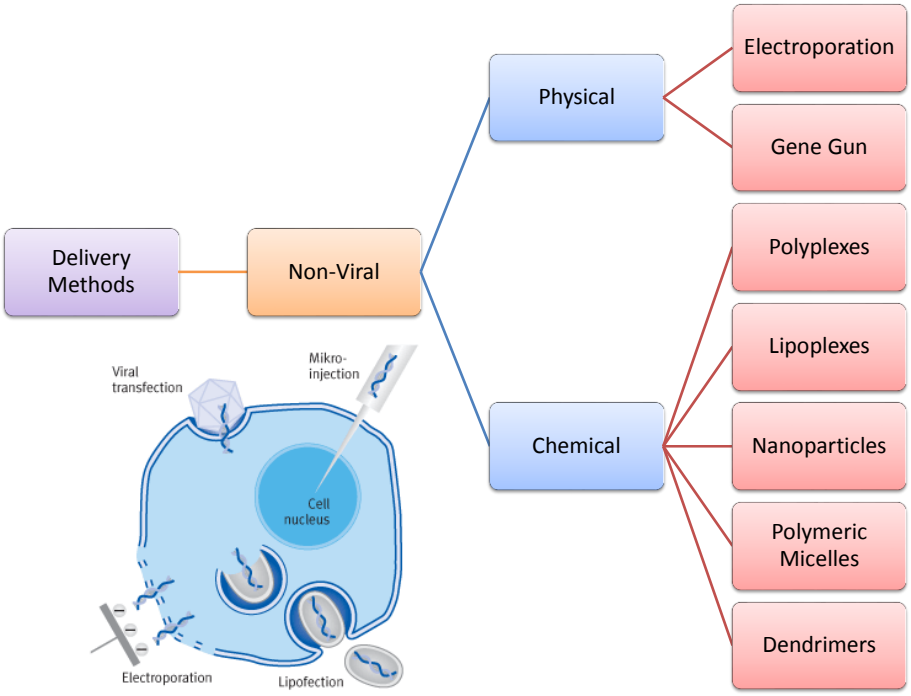
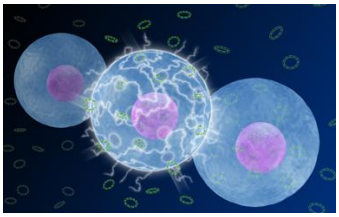


Table 1.1. Scientific milestones that impacted the field of non-viral gene therapy.

Scientific Milestone	Year	Refs.
First Liposome Based DNA Delivery Patent filed	1983	1
First publications describing the use of cationic lipids to transfect cells	1987–89	2–4
Demonstration that "Naked DNA" can Transfect muscle cells *in vivo*	1990	5
First human clinical trial conducted for development of melanoma cancer vaccine using cationic lipid formulated plasmid DNA	1996	6, 7
First indications of clinical efficacy demonstrated for treatment of Chronic Limb Ischemia following IM administration of VEGF Naked pDNA	1996	8, 9
Electroporation yields order of magnitude increase in gene expression following local administration	1998	10
Aqua Health (Novartis) anti-viral vaccine for salmon receives approval in Canada.	2005	
Successful demonstration of efficacy for treatment of chronic limb ischemia following IM administration of pDNA expressing hepatocyte growth factor.	2007	
Merial receives conditional USDA approval of canine melanoma therapeutic genetic vaccine.	2008	

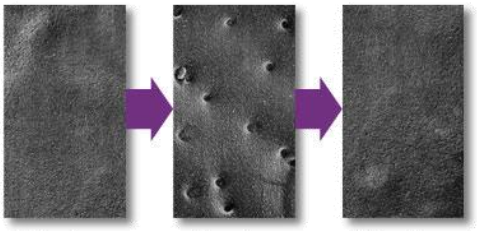
Electroporation

- Momentary exposure of cells suspended in DNA solution to a high electrical field



The phenomenon of electroporation

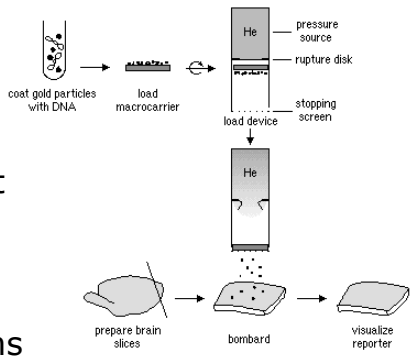
- Advantages:
  - High transfection efficiency
- Disadvantages:
  - Damage of a significant number of cells



Gene Gun

- Also called Biolistic transformation

- Advantages:
  - Can be used in any cell type
- Disadvantages:
  - Damage of a significant number of cells
  - Low transfection efficiency
  - Reproducibility problems



Nanoparticles : Goals

- More specific drug targeting and delivery
- Reduction in toxicity while maintaining therapeutic effects
- Greater safety and biocompatibility
- Faster development of new safe medicines

Table 2 Chemicals under investigation for drug delivery

Albumin	Damascelli et al 2003
Cetyl alcohol/polysorbate	Koziara et al 2004
Chitosan	Dyer et al 2002; Huang et al 2004
Gelatin	Cascone et al 2002
Gold	Hainfield et al 2004; Paciotti et al 2004
Hydrogels	Gupta and Gupta 2004
Magnetic iron oxide	Gupta and Gupta 2005
Methoxy poly(ethylene glycol)/poly(ε-caprolactone)	Kim et al 2003
Polyalkylcyanoacrylate composites	Alyautdin et al 1997; Kreuter et al 2003
Poly(D,L-lactic-co-glycolic)acid (PLGA)	Panyam et al 2002; Weissenbrock et al 2004
Solid lipid formulations	Muller et al 2000; Wissing et al 2004

Nanoparticles

- Polyplexes
- Lipoplexes
- Polymeric NPs
- Polymeric micelles
- Dendrimers

Table 1 Overview of nanoparticles and their applications in Life Sciences

Particle class	Materials	Application
Natural materials or derivatives	Chitosan Dextrane Gelatine Alginates Liposomes Starch	Drug/Gene delivery
Dendrimers	Branched polymers	Drug delivery
Fullerenes	Carbon based carriers	Photodynamics Drug delivery
Polymer carriers	Polylactic acid Poly(cyano)acrylates Polyethyleinemine Block copolymers Polycaprolactone	Drug/gene delivery
Ferrofluids	SPIONS USPIONS	Imaging (MRI)
Quantum dots	Cd/Zn-selenides	Imaging In vitro diagnostics
Various	Silica-nanoparticles Mixtures of above	Gene delivery

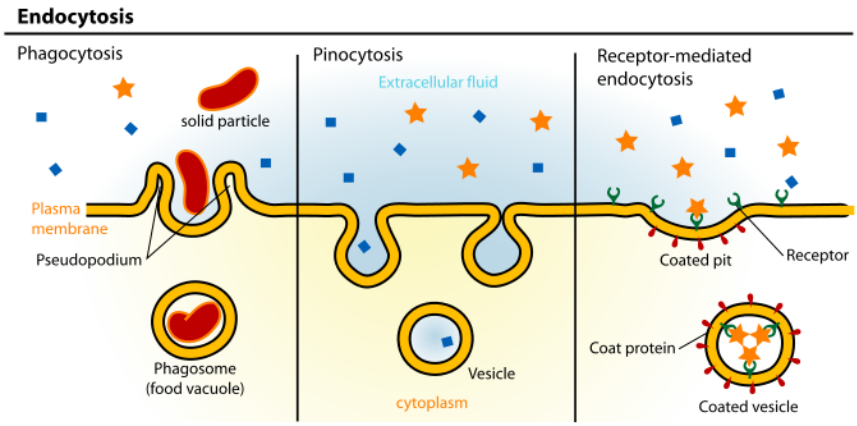
Co-precipitation

- Binding of DNA (-ve) into macromolecular complexes e.g. Calcium Phosphate crystals (+ve). Uptake by ***endocytosis***.

- Advantages:
  - Inexpensive and simple to perform
  - High transfection efficiency

- Disadvantages:
  - Cytotoxicity

Endocytosis



Main issue using nanoparticles

- Drug incorporation and release
- Formulation stability and shelf life
- Biocompatibilty
- Biodistribution and targeting
- functionality

De Jong & Borm, 2008.

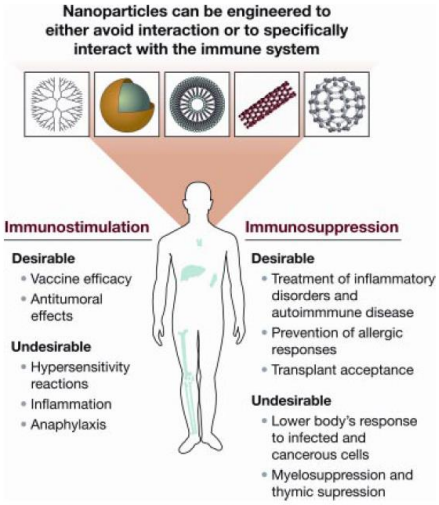


FIG. 1. Nanoparticle interactions with the immune system. Nanoparticles' effects on the immune cells may benefit treatment of disorders mediated by unwanted immune responses and enhance immune response to weak antigens. On the other hand, undesirable immunostimulation or immunosuppression by nanoparticles may result in safety concerns and should be minimized.

Zolnik et al., 2010

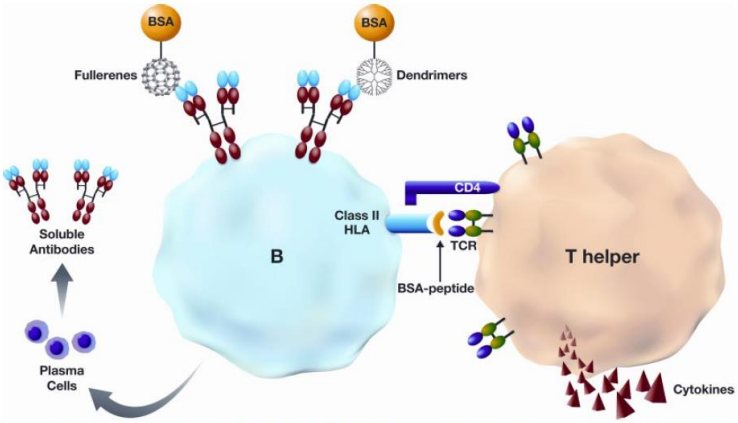


FIG. 2. Hypothetical model of nanoparticle antigenicity. Nanoparticles (dendrimers and fullerenes) may not be antigenic unless they are bound to a protein carrier such as BSA. BSA-nanoparticle conjugate is endocytosed by B cells, and the protein is digested inside the cell. B cells then may present BSA peptides on their human leukocyte antigen-class II molecules to T-helper cells. Both cells become activated, resulting in production of cytokines by T lymphocytes and antibodies directed to nanoparticles by plasma B cells. The protein carrier is indispensable for T cell activation. Antibodies to fullerenes and dendrimers are produced by different B cells. For simplicity of the figure, only one B cell is shown. TCR, T cell receptor.

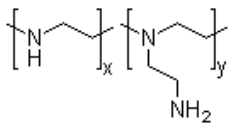
Zolnik et al., 2010

Polyplexes

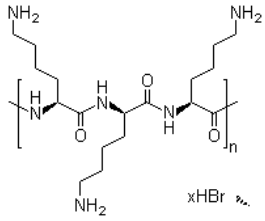
- They are complexes of cationic polymers with nucleic acids
- Polyplex formation is regulated by electrostatic interaction, which is affected by:
  - pH of the media
  - Ionic strength
  - Cationic density

Polyplexes

- Examples:
- Polyethylenimine (PEI)
- Poly-L-Lysine (PLL)

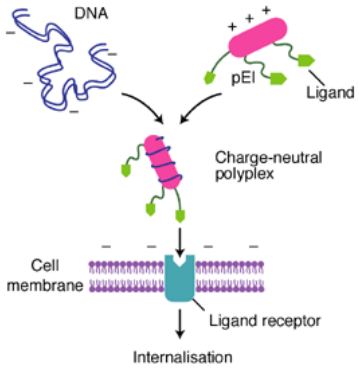


PEI



PLL

Polyplexes



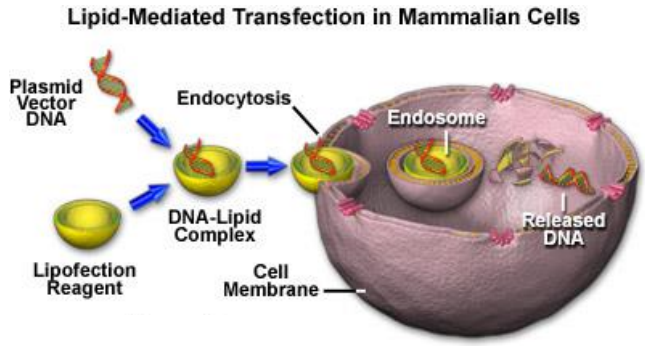
Polyplexes

- Advantages:
  - Easy to formulate
  - Simple chemical modification
- Disadvantages:
  - Cytotoxicity
  - Stability problems

Lipoplexes

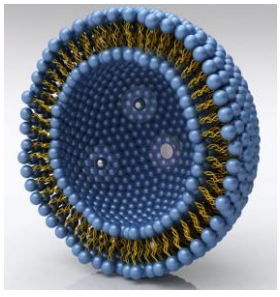
- They are complexes of cationic lipids or liposomes with nucleic acids
- Lipoplex formation is regulated by electrostatic interaction and lipid constituents of the carrier
- Transfection using lipoplexes is known as ***Lipofection***

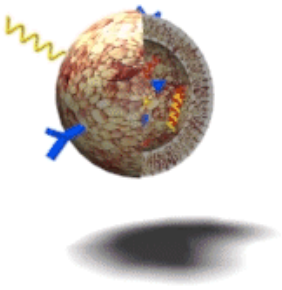
Lipofection



Lipoplexes

- Examples:
- Lipofectin
- Lipofectamine



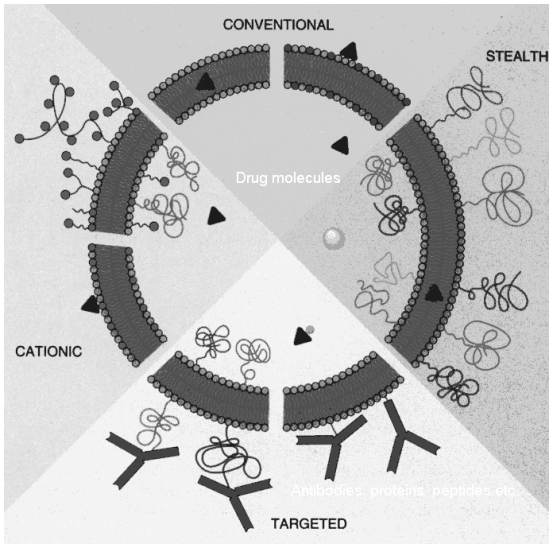


Lipoplexes

- Advantages:
  - Versatility
  - Protection of DNA
  - Fusion with cell membrane
  - Biocompatibility

- Disadvantages:
  - Labor intensive
  - Expensive
  - Stability
  - Clearance by RES

Liposome versatility



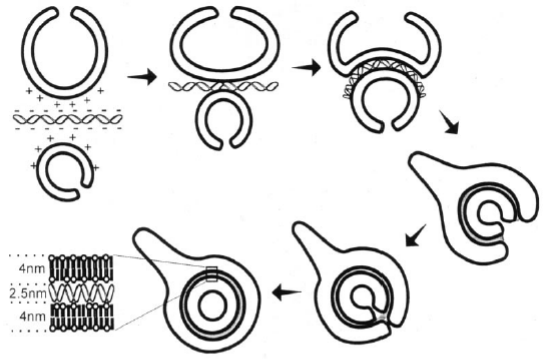


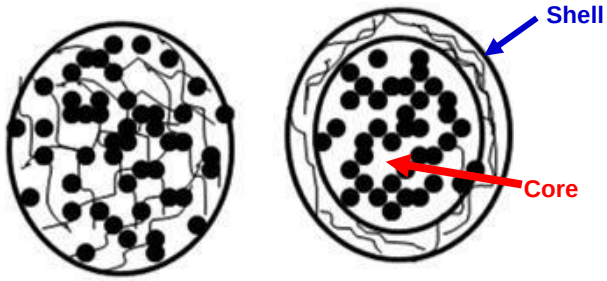
Fig. 2. Proposed model showing cross-sections of extruded DOTAP:Chol liposomes (BIVs) interacting with nucleic acids. Nucleic acids adsorb onto a BIV via electrostatic interactions. Attraction of a second BIV to this complex results in further charge neutralization. Expanding electrostatic interactions with nucleic acids causes inversion of the larger BIV and total encapsulation of the nucleic acids. Inversion can occur in these liposomes because of their excess surface area, which allows them to accommodate the stress created by the nucleic acid-lipid interactions. Nucleic acid binding reduces the surface area of the outer leaflet of the bilayer and induces the negative curvature due to lipid ordering and reduction of charge repulsion between cationic lipid headgroups. Condensation of the internalized nucleic acid-lipid sandwich expands the space between the bilayers and may induce membrane fusion to generate the apparently closed structures. The enlarged area shows the arrangement of nucleic acids condensed between two 4 nm bilayers of extruded DOTAP:Chol.

Polymeric Nanoparticles

- Nanoparticles made out of polymers
- Natural polymers:
  - Chitosan
  - Albumin
  - Gelatin
- Synthetic Polymers:
  - Polylactic acid (PLA)
  - Polyglycolic acid (PGA)
  - Poly(lactic-co-glycolic acid) (PLGA)

Polymeric Nanoparticles

- Polymeric NPs can be prepared to become ***Nanospheres*** or ***Nanocapsules***

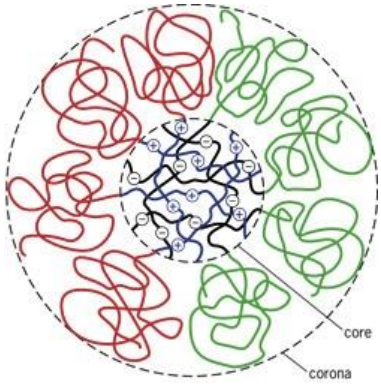


Polymeric Nanoparticles

- Advantages:
  - Biocompatibility
  - Biodegradability
  - Loading of more than one gene or drug
- Disadvantages:
  - Tedious preparation
  - Degradation of DNA during preparation
  - Can't be easily modified

Polymeric Micelles

- Amphiphilic polymer chains that self-assemble into nano-sized spherical structures in aqueous medium

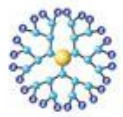
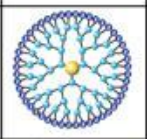


Polymeric Micelles

- Advantages:
  - Biocompatibility
  - Biodegradability
  - Easy to prepare
  - Chemical modification is easy
- Disadvantages:
  - Highly unstable
  - Must be made fresh

Dendrimers

- Highly-branched polymeric macromolecules

Generation	G0	G1	G2	G3	G4
# of Surface Groups	3	6	12	24	48
Diameter (nm)	1.4	1.9	2.6	3.6	4.4
2D Graphical Representation					
3D Chemical Structure View					

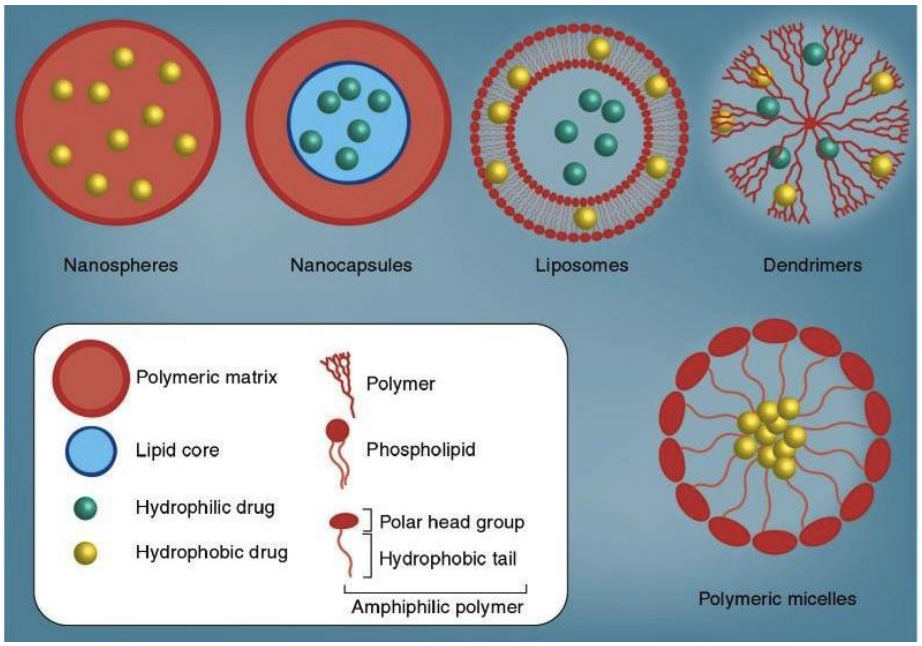


Table 4 Toxicity of engineered and combustion (nano) particles as illustrated by their most unique adverse effects in vivo and in vitro

Description of finding, in vivo	Particle types
NPs cause pulmonary inflammation in the rat	All PSP
Later studies show that inflammation is mediated by surface area dose.	SWCNT, MWCNT
NPs cause more lung tumors than fine particles in rat chronic studies. Effect is surface area mediated	PSP only
NPs cause progression of plague formation (ApoE -/- mice)	SWCNT, PM2.5
NPs affect immune response to common allergens	Polystyrene, CB, DEP
NsP can have access to systemic circulation upon inhalation and instillation.	Specific NP, dependent on surface coating
Description of finding, in vitro	
NPs cause oxidative stress in vivo and in vitro, by inflammatory action and generation of surface radicals.	PSP, NP general, CNT
NPs inhibit macrophage phagocytosis, mobility and killing	CB, TiO_2
NPs cause platelet aggregation	PM, SWCNT, fullerenes, latex-COOH surface
NPs exposure adversely affects cardiac function and vascular homeostasis	PM, SWCNT
NPs interfere with Ca-transport and cause increased binding of pro-inflammatory transcription factor NF-kB	CB (<100 nm), ROFA, PM2.5
NPs can affect mitochondrial function	Ambient NP,
NPs can translocate to the brain from the nose	MnO_2, Au, carbon
NPs do affect rolling in hepatic tissue	CB