Targeted Drug Delivery with Nanoparticles

- Introduction
- Classification drug targeting technologies
- Physical chemical features
- Routes of administration and pharmacokinetics
- Toxicity and safety aspects
- Mechanisms of intracellular delivery and targeting
- Triggered release
- Disease examples: cancer
- Human translation
- Regulatory aspects
- Concluding remarks
I. Introduction

• What is targeted drug delivery?
• Aim: selective drug disposition
  → improved drug efficacy
  → improved drug safety
  → improved therapeutic index
  → prolonged drug action
  → enabling use of otherwise ‘impossible’ drug
Fields of targeted drug delivery?

1) **Improve therapeutic index** (improve efficacy and reduce toxicity)
   - cancer medications
2) **Reaching difficult organs**
   - brain, retina, tumours
3) **Intracellular drug delivery**
   - oligonucleotides, intracellular proteins, genes
4) **Prolonging drug response**
   - drug retention and release are prolonged (e.g. retina)
II. Classification drug targeting technologies

• Various technologies and division lines
• Biological – synthetic
• Processed – self-assembling
• Passive targeting – active targeting
• Extracellular or intracellular drug release
• Local or systemic drug delivery
• Drug covalently or non-covalently bound
• Biodegradable, biocompatible
• Hybrids and combinations
Targeted drug delivery systems

**DRUG NANOCRYSTALS**

**LIPID BASED SYSTEMS**

- * micelles
- * liposomes
- * hexosomes and cubosomes
- * solid lipid nanoparticles, nanoemulsions
- * lipid complexes

**POLYMER BASED SYSTEMS**

- * polymeric nanoparticles
- * polymeric micelles
- * polymeric conjugates
- * dendrimers
- * polymersomes
- * polymer complexes

**INORGANIC MATERIALS**

- * silicon, silica
- * metals
- * carbon nanotubes

**MICROVESICLES**

**ANTIBODIES**
Nanostructures Based on Self-Association of Lipid Like Molecules

Molecular shapes: cone, rod, inverted cone
Methods: SAXS, EM, phase diagrams
Surfactant micelles

Micelles are small - typically about 10 nm in diameter
Monolayer

Micelles have high CMC (often at mM range)
→ after injection concentration falls below CMC
→ micelles disassemble
→ failure in targeted drug delivery

Cross-linking could stabilize the micelles, but this generates other problems

ABANDONED APPROACH
Liposomes

- Size 25 - 10 000 nm, bilayer(s)
- CMC in nM range
- Stability depends on the lipids
  - PC, DOPE, CHOL
  - Alkyl chain length
    (phase transition temp)
- LUV, MLV, SUV; REV
- Self-assembling
- Manufacture:
  - extrusion
  - REV-process
  - Detergent dialysis
  - Szoka’s filtration method
- drug encapsulation
  - hydrophilic, lipophilic
  - remote encapsulation
    (doxorubicin)

PEGylation, targeting
Mature field, products
Virosomes failed
Solid lipid nanoparticles

- Spherical in shape
- Solid lipid core stabilized by a surfactant
- Core lipids: fatty acids, acylglycerols, waxes, and mixtures.
- Stabilizers: phospholipids, sphingomyelins, bile salts (sodium taurocholate), and sterols (cholesterol)
- For lipid soluble drugs, size about 100 nm
- Processing: sonication, microfluidization
Polymeric nanoparticles

Processed, nanoprecipitation etc

Size range like in liposomes

**Polymers:** PLGA, PLA, cyanoacrylates

**Functionalization:** PEGylation, targeting moieties

“Mature field”
Polymersomes

Amphipathic block copolymers

Hydrophobic blocks in the membrane
Hydrophilic blocks oriented outwards

Hydrophobic and hydrophilic drugs
Versatile system, still relatively new

Typically 100 nm
Polymeric micelles

Polymeric micelle can be formed spontaneously in water solution or in the presence of the drug.

CMC in µM range

Lipid soluble drugs partition to the core.

Stability in plasma?
Widely studied.
Mesoporous silicon and silica

Mesoporous (5-30 nm pores)

Sol-gel method, spray drying, template synthesis

Drug loaded to the pores; control of release?

Widely studied, fate of materials?

Drug release
Viruses

• Widely studied as gene delivery agents
• Safety, production
• Various types (adenovirus, AAV, Herpes simplex)
• Viral mechanisms as source of inspiration
• Virosomes
III. Physical chemical features
Size

- Depends on the system: 1 - 1000 nm
- Polydispersity can be problematic because distribution is size dependent
- Smaller particles $\rightarrow$ better tissue penetration, but smaller drug loading
- Large particles in the sample may contain large fraction of the dose
  - Volume of 1000 µm particle is 1000x bigger than the volume of 100 nm particle
Surface characteristics

• Charge interactions
• Hydrophobic surface $\rightarrow$ aggregation and adherence problems
• Steric stabilization (PEG, HPMA, hyaluronic acid)
• Targeting moiety and its quantity
  – Typically targeting moiety is attached in the tip of PEG or other stealth coating
• Stability
  – Shelf life
    • Covalent links, aggregation
    • Medium; ionic strength and pH effects
  – Freeze drying, reconstitution
  – In plasma
    • Protein adherence, changes in the behavior
    • Drug release
    • Disassembly (plasma components, CMC issues)
IV. Routes of administration and pharmacokinetics

• Per oral
  – Tight junctions in the intestinal wall inhibit absorption of nanoparticles
  – Oral targeted drug delivery requires small molecular prodrug approach
  – Nanotechnologies may increase per oral bioavailability

• Local injections
  – Ocular, intra-tumoural, during surgery (cardiac, brain)
  – Prolonged retention, improved efficacy

• Intravenous injection
  – The most common route in targeted drug delivery
Distribution

Drugs should escape from blood stream to access the target cells. Targets: extracellular, intracellular.

Distribution is controlled by the blood flow and the barriers between the blood and tissues. Barriers are different.
Nanoparticulate distribution (i.v. injection)

distribution is controlled by the blood flow and the barriers between the blood and tissues

*Lung capillaries*
aggregates, no good

- **Liver, spleen**
  - reticuloendothelial system
  - phagocytosis in macrophages
  - even 10 µm particles
  - opsonization of proteins (protein corona facilitates RES uptake)

- **Tumours**
  < 100 nm nanoparticles extravasate

- **Difficult sites with tight endothelia**
  - brain, retina
  - specialized transport systems needed to overcome BBB and BRB
Elimination

- Kidney pore size 4.5-5 nm
- Positive charge

Prevents elimination
Renal elimination of macromolecules and nanoparticles

- Glomerular filtration (mw less than 50 kDa), nanoparticles not eliminated through kidney
- Polymer size would allow tumour localization and
- Evasion of glomerular filtration

- a) PVA: 13 kDa
- b) PVA 580 kDa
- c) glomerular pore
- d) Large endothelial junction (healthy tissue)
- e) Typical pore in tumour
- f) Very large endothelial junction (cancer tissue)
Hepatic elimination

- Nanoparticles do not eliminate via kidney → accumulation in the liver
- Leaky vessels in the liver → macrophages take up the nanoparticles → degradation
- Opsonization facilitates uptake
- Hepatic targets
Blood circulation

• Protein adherence $\rightarrow$ protein corona around nanoparticles

• Plasma contains 3000 proteins; can be analyzed
Protein corona formation

Often 50-125 proteins adhere (e.g. silica NP)

Protein corona may change during NP distribution

PK is modified

Conformation of the adhered proteins may have impact on NP distribution.

Targeting ability may be lost in vivo.
Stealth coating

* polyethylene glycol – improves shelf-life

PEG about 2-5 kDa
Minimizes protein interactions → prolongs the circulation time in plasma from 1-2 hours → 24-48 hours
PEG is controversial

Positive

Prolonged retention → better chances for tissue distribution

Negative

Accelerated blood clearance (ABC) in repeated administration - IgM against PEG formed. Sometimes they exist before treatment. NP, liposomes, protein conjugates

Not always reduced protein adherence.

May decrease cellular uptake.

Alternative approaches needed.
Successful nanomedicine must overcome several steps.
Distribution in the body

Lehtinen et al., PlosONE 2012
V. Toxicity and safety aspects

In vitro tests with cell cultures to screen materials

MTT, LDH, PI etc tests; Interleukin secretion
Complement activation

In general cationic polymers and lipids are more toxic than neutral or anionic materials

In vitro tests at various concentrations – exposure in vivo should be estimated

Modulation of drug toxicity
  * more drug in liver and spleen → toxicity
In vivo toxicology

Data is sparse; much more research on efficacy and delivery

Long term toxicity needs to be determined before clinical acceptance

Some toxicity aspects:
* influence of coatings
* inorganic materials – how they are cleared from the body?
* PEG – ABC effect; even 25% of patients may have PEG antibodies before treatment (from cosmetics, pharmaceuticals)
* PLGA – local acidification – inflammatory responses
* cationic materials tend to have toxicity in cells, bind many surfaces
* protein corona and toxicity
* carbon nanotubes show asbetosis type toxicity
VI. Mechanisms of intracellular delivery and targeting

Drug target: intracellular or extracellular

Drug penetration into the cells: Yes/no

Intracellular target and no drug penetration to the cells as such → Intracellular targeted delivery is requirement

Targeted delivery to the
   1) extracellular space in target tissue → drug release locally
   2) into the target cells in the tissue
Drug Targeting with Nanosystems
Intracellular delivery

Studied in cell culture

Cargo and/or delivery system labeled with fluorophores

Localization investigated with confocal microscope

Important steps

Binding on the cell surface (targeted or non-targeted)

Cellular uptake (phagocytosis, endocytosis: cavelae or clathrin mediated)

Endosomal escape (pH acidification; fusion with endosomal membrane needed for siRNA, DNA)

Lysosomal localization (drug release)
Targeting systems
Peptides (phage display library screening)
Aptamers (SELEX search)
Antibodies (phage display library screening)
Hyaluronic acid (CD44)
Small molecules
Nuclear localization signal peptide
Magnetic steering

The main benefit is NOT getting more drug to the tissue, but rather to improve *cellular delivery* and *retention*.

**Requirements:**

1) selectivity to the target cells
2) high affinity
3) chemically feasible
VII. TRIGGERED RELEASE

Drug release from targeting formulation is needed for drug activity.

Ideally, release should be triggered at the site of action.

Triggering principles:

1) **Endogenous triggering.**
   - Lower pH in endosomes and lysosomes (pH sensitive liposomes, polymeric prodrugs)
   - Enzyme activity in target cells (polymeric conjugates)
   - Heat sensitive release (inflammation, tumours - higher temp. (liposomes)

2) **Exogenous triggering.**
   - Magnetic release (heating iron nanoparticles \( \rightarrow \) release)
   - Ultrasound based release
   - Light activated release
Passive and active targeting to the tumours

EPR = Enhanced Permeation and Retention Effect
Escape from blood vessels to tumours:

< 100 nm

Targeting to

1) neovessels in tumours
2) tumour cells
3) extracellular matrix in tumour

Tumour penetration enhancement peptides (iRGD)
losartan
hyaluronidase

IX. Clinical aspects

Number of nanomedicines and targeted drug delivery systems is low.

Almost all their clinical trials are related to cancer.

Animal-to-man translation in cancer field is poor – this may reflect also to nanomedicines.

Best success with Ab-drug conjugates.

Potential is high anyway.
X. Regulatory aspects

FDA and EMA do not accept new excipients or new drug delivery systems
→ They approve new drugs (including excipients etc)
→ Nanomedicine is evaluated together with a drug (new drug or old drug)

![Diagram showing risk levels and development time]

- Risk levels: H, M, L
- Development time: <2, 2-5, 6-7 years

Research (3 years)
- synthesis, testing,
- Analytics
- specifications
Pilot plant, tox (3 years)
Total 6-7 years DMF
Then, formulation with API → approval
→ Monograph to pharmacopeia (total 10 years)
Conclusions

Potential of targeted drug delivery
- potential to improve old drugs
- enabling technology to new drugs
- from some biologics is essential
- improving delivery to difficult targets
- technology expanding – although plenty of hype!

Limitations
- regulatory process
- toxicity issues
- true benefits must be shown
- differs from the ‘normal’ industrial development