Strategies for Efficient Delivery and Effective Intracellular Release by Polymeric Micelles

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Supramolecular segregation of therapeutic agents can increase their delivery with reduction of the total administered dose

Liposome

- Hydrophilic environment

- Reduced renal excretion
- Reduced catabolism
- Protect unstable molecules
- Can allow targeting to diseased tissues (active targeting/passive targeting)

Micelle

- Lipophilic environment

- Allow the use of not water soluble drugs
**Systemic administration of drug loaded micelles: how to get an efficient and effective delivery to diseased tissues**

1. **Transport via systemic circulation**
   - ✔ Sufficient stability of loaded micelles

2. **Escape from immune system control**
   - ✔ A small size of the carrier reduces recognition by the mononuclear phagocytic system in the spleen and in the liver
   - ✔ Highly hydrated particles have antifouling properties with protein absorption (opsonization) reduction. This inhibits the recognition by macrophages

3. **Extravasation from vascular system**
   - ✔ Enhanced permeability and retention effect (EPR) **Passive Targeting**

4. **Cells internalization**
   - ✔ Activated uptake by endocytosis

5. **Release of micelles’ contents**
   - ✔ Upon cells internalization the micelles should be destabilized to release the drugs

6. **Cytoplasm availability of the delivered drug**
   - ✔ Endosmolytic properties of the carrier
Molecular surfactants vs. Polymeric surfactants

Typical detergent
(amphiphilic molecule)

Sulfate head
Aliphatic tail

Detergent micelle

Above the critical micelle concentration (CMC)

Self-assembling in water

We can represent a detergent as a hydrophilic (water loving) “head” and... ...
...a hydrophobic (water fearing) tail

Thermodynamically governed process
Low kinetic stability
Molecular surfactants vs. Polymeric surfactants

Amphiphilic block-copolymer

Hydrophilic block

Lipophilic block

PMMA

Kinetic stability

Polymeric Micelle
(highly stable core-shell spherical particle)

Hydrated shell
(Confers thermodynamic stability)

Morpholine substituents allow multiple effects stimulated by pH changes

Self-assembling in water

-self-assembling in water

Hydrated shell
(Confers thermodynamic stability)
Polymeric micelles as delivery vessels

Amphiphilic block-copolymer

Hydrophilic block

Lipophilic block
PMMA

Kinetic stability

Polymeric Micelle (highly stable core-shell spherical particle)

Self-assembling in water

Lipophilic agents (fluorophores, drugs...)

Hydrated shell (Confers thermodynamic stability and gives antifouling properties)
Small size and high kinetic stability of the particles

Hydrophilic nanoparticles below 35 nm show much less uptake in the spleen and in the liver (RES) by monocytes and macrophages.

![AFM height image and respective profiles of the coloured sections](image)

<table>
<thead>
<tr>
<th>Micelles-Dye</th>
<th>Diameter (nm)</th>
<th>Z Potential (mV)</th>
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</thead>
<tbody>
<tr>
<td>M[Empty]</td>
<td>25</td>
<td>+30</td>
</tr>
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<td>M-4</td>
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<tr>
<td>M-9</td>
<td>40</td>
<td>+21</td>
</tr>
</tbody>
</table>

DLS average diameter and Z potential of empty and fluorophores loaded micelles in deionised water (pH 5-6)

The behaviour of the deposited micelles to form agglomerates without nucleus coalescence reveals their high kinetic stability and ensure the integrity of the supramolecular nanostructure in the cell culture media.

![AFM peak force mode images of height (a), peak force error (b) and phase image (c)](image)
Low bioadhesion on highly hydrated surfaces

Mostly used polyethylene glycol (PEG)

Hydration Layer

Hydrophilic Polymers

Zwitterionic Polymers

Proteins

Generally Polybetaines

Antifouling or anti non-specific protein adsorption properties

Low bioadhesion on highly hydrated surfaces

Amphiphilic block-copolymer

Hydrophilic block

Lipophilic block

PMMA

Lipophilic agents (fluorophores, drugs...)

Kinetic stability

Self-assembling in water

Hydrated shell (Gives stealth properties)

Highly stable core-shell spherical particle
Extravasation from vascular system

Enhanced Permeability and Retention Effect (EPR)

Passive Targeting

- Defective or “leaky” vasculature in tumors
- Micelles carried by blood stream enter into tumors passively
- Angiogenic blood vessels in tumors
- Normal vasculature display continuous and well formed endothelial cells junctions
- The drainage in tumor tissues is reduced

Lymphatic drainage
Cells internalization of nanoparticles

Different types of endocytosis

A. Phagocytosis
B. Clathrin-mediated endocytosis (CME)
C. Caveola-mediated endocytosis (CvME)
D. Macropinocytosis
E. Other clathrin- and caveola-independent endocytosis

Cell membrane

Charge interaction

The studied particles increase their outer positive charge in the acidic environment of the tumor.
Cells internalization of loaded nanoparticles

Oligothiofene based fluorophores

FRET paired fluorophores
Förster Resonance Energy Transfer (FRET) consists in an energy transfer from the excited state of the donor chromophore to the acceptor chromophore through a nonradiative dipole–dipole coupling. The efficiency of this energy transfer is inversely proportional to the sixth power of the distance between donor and acceptor, making FRET extremely sensitive to small changes in distance.

Quenched emission of the green fluorophore
Triggered release of micelles’ contents

The increased pH within endosomes destabilizes the micelles releasing their contents.
The disappearing of FRET after endocytosis reveals the release from micelles

Images obtained by merging the two separate emission at confocal light scanning microscope of the two FRET paired green and red fluorophores

It is clearly visible that the two fluorophores (TFs) are colocalized but the green color is predominant as it happens when they are in solution. This means that, although the fluorophores reside in the same subcellular compartment, the FRET phenomenon is not occurring thus demonstrating the release of the micelles’ contents. The naked TFs (in DMSO) appear separated upon cells internalization.
Does cellular uptake mean real intracellular release?

Once internalized the nanoparticles and their contents have to be delivered to the cytoplasm before being expelled again by exocytosis or being destroyed by lysosomes.
Does cellular uptake mean real intracellular release?
Endosmolytic release by “proton sponge effect”

The protonation of the nitrogens exerts a triple function:

1) Stimulate the endocytosis by charge interaction
2) Micelles destabilization and release of their contents
3) Water entry into the endosomes with consequent membrane rupture and delivery to the cytoplasm
Synthesis of the amphiphilic block copolymer

RAFT agent

1) GMA
2) MMA

Poly(glycidyl methacrylate)-b-poly(methyl methacrylate)

RAFT removal with ends functionalization

Post-polymerization process with morpholine
The developed process enables a multitude of different materials specifically functionalized and able of precise functions.

In particular this process provides polymeric materials for:

- Diagnostic agents delivery
- Therapeutic agents delivery
- Gene delivery
- Active and/or passive targeting
- Anti non-specific protein absorption properties
- Endosmolytic properties

Theranostic approach