

Thermosensitive Micelles for Targeted Drug Delivery

Wim E. Hennink, Osamu Soga, Christianne Rijcken, Tina Vermonden, Roberta Censi, Gert Storm, Rene van Nostrum

Department of Pharmaceutics
Utrecht Institute for Pharmaceutical Sciences
Utrecht University
The Netherlands

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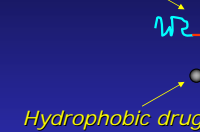


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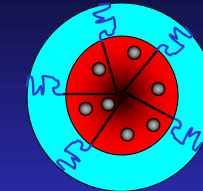
UIPS Utrecht Institute for
Pharmaceutical Sciences

Block copolymers as drug carrier

Amphiphilic block copolymer



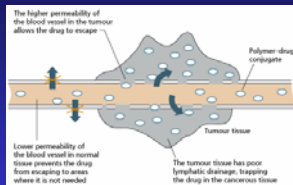
+ water



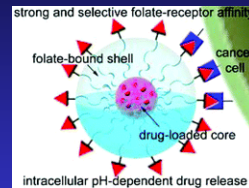
"DRUG CARRIER"

- Incorporation of hydrophobic drugs
- Long circulation in the blood stream after i.v. administration
- Accumulation in e.g. tumor tissues

Block copolymer micelles: Drug targeting

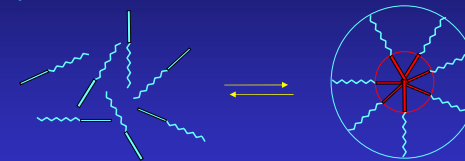
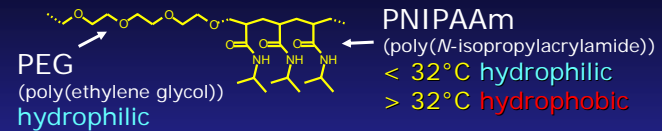


Passive targeting



Active targeting

PNIPAAm-*b*-PEG block copolymer



Room Temperature

Body Temperature

Simple micelle formation by heating (Pro)

Non-biodegradable and no controlled release (Con)

poly(HPMAm-lactate): a degradable thermosensitive polymer

Poly(*N*-(2-hydroxypropyl) methacrylamide) (pHPMAm, non-toxic drug carrier)

poly(HPMAm-monolactate) (pHPMAmML) poly(HPMAm-dilactate) (pHPMAmDL) poly(HPMAm-monolactate-co-HPMAm-dilactate) (pHPMAmML-co-HPMAmDL)

Radical polymerization (at 70°C for 24hrs in dioxane)

Static light scattering measurements

Intensity

Temperature (°C)

HPMAmML : HPMAmDL = 50:50 (molar ratio)

25°C 37°C

poly(HPMAm-lactate) is a thermosensitive polymer with LCST properties

Effect of polymer composition on cloud point

Cloud point (°C)

Mol % of HPMAm-monolactate

insoluble

soluble

- CP can be tailored by copolymer composition
- CP is expected to increase in time

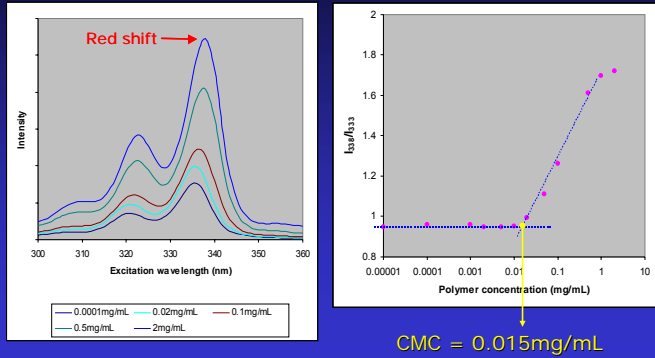
Synthesis of p(HPMAm-dilactate)-*b*-PEG block copolymer

PEG₂-ABCPA + HPMAm-dilactate (HPMAmDL)

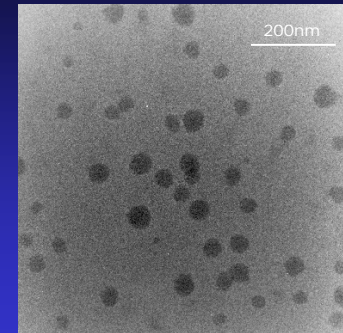
Radical polymerization (70°C in ACN)

poly(HPMAm-dilactate)-*b*-PEG block copolymer (p(HPMAmDL-*b*-PEG))

CMC determination (partition of pyrene into micelles)



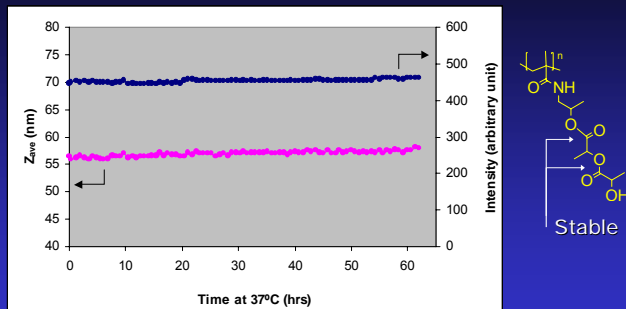
Cryo-EM results



Polymer:
pHPMAmDL(13600)-*b*-PEG
10mg/mL

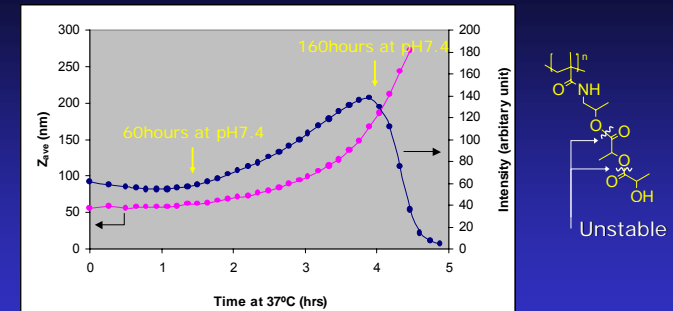
Spherical micellar
structures with
narrow size distribution
(25-50nm, Ave=40nm)

Micelle stability (pH5.0)



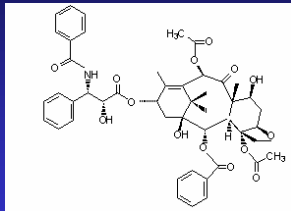
Micelles are stable under conditions where hydrolysis of lactate side groups is minimal

Micelle stability (pH9.0)



Destabilization of micelles due to hydrolysis of lactate side groups (increase of CP above 37°C)

PTX loading into pHPMAmDL-*b*-PEG block copolymer micelles

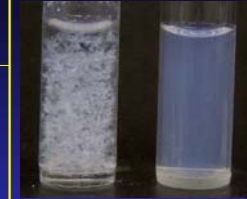


Paclitaxel (PTX) $MW = 853.9$
 Highly potent anticancer drug
 but difficult to formulate because of
 its strong hydrophobicity
 (solubility in water = $0.3 \mu\text{g/mL}$)

Taxol[®] (PTX formulated in Cremophor EL : ethanol = 1:1)
 is currently used but Cremophor EL is
 associated with adverse effects

Results of PTX loading

	PTX + buffer	PTX + PEG	PTX + pHPMAmDL - <i>b</i> -PEG
PTX feed (mg/mL)	1.0	1.0	1.0
Polymer (mg/mL)	-	9.0	9.0
PTX solubilized (mg/mL)	0.0015	0.0012	0.96
% dissolved	0.15%	0.12%	96%
Z_{ave} (PD)	-	-	60 nm (0.06)

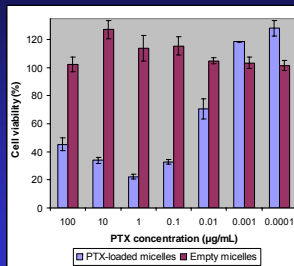


PTX +
PEG PTX +
pHPMAmDL
-*b*-PEG

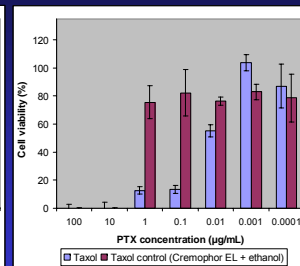
Strong solubility enhancement of PTX by
 pHPMAmDL-*b*-PEG
 (solubility $\sim >1,000$ -fold greater than in water)

In vitro cytotoxicity (B16-cells)

PTX-loaded micelles



Taxol



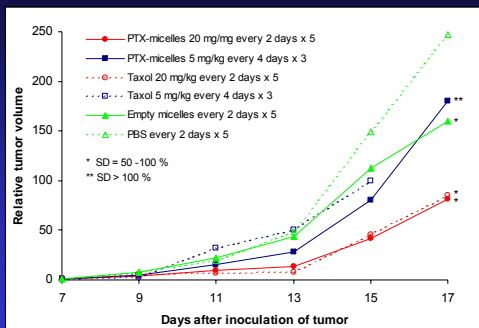
- Comparable antitumor effect
- Micelles are far less toxic than Cremophor EL

In vivo antitumor efficacy of PTX-loaded micelles

(B16 melanoma bearing mice, i.v. injection)

- Group 1: PTX-loaded micelles 20mg/kg, every 2 days x 5
- Group 2: PTX-loaded micelles 5mg/kg, every 4 days x 3
- Group 3: Empty micelles every 2 days x 5
- Group 4: Taxol 20mg/kg, every 2 days x 5
- Group 5: Taxol 5mg/kg, every 4 days x 3
- Group 6: PBS 200 μL , every 2 days x 5

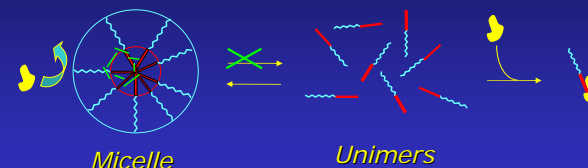
In vivo antitumor efficacy



- Clear dose response
- PTX-Micelles are comparable with Taxol

in vivo: (empty) micelles

Polymer adsorption to proteins ?

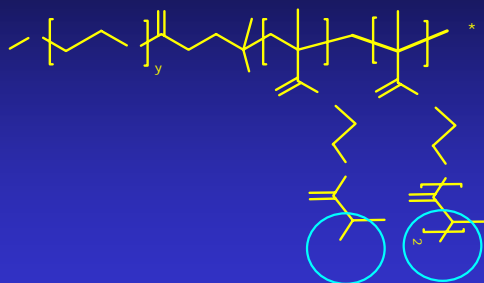


How to prevent micelle destabilization?

Approach: crosslinking the core of the micelles

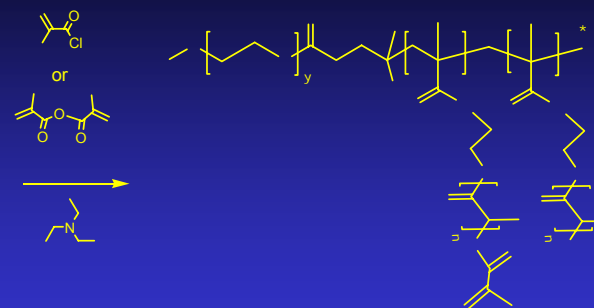
CCL

Core Crosslinked Micelles



CCL

Crosslinkable Group



CCL

Photopolymerization

- Irgacure® 2595
- 10 min UV

NCL 67 ± 8 nm

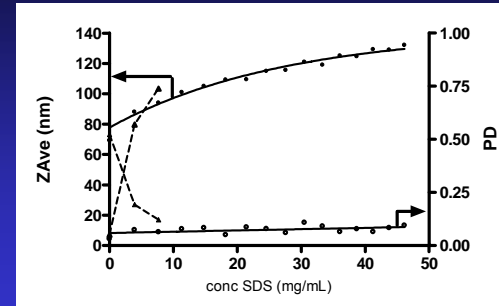


CCL 68 ± 7 nm

CCL *in vitro*

Physical Stability

addition sodium dodecyl sulphate



- - NCL; — CCL

CCL *in vivo* biodistribution

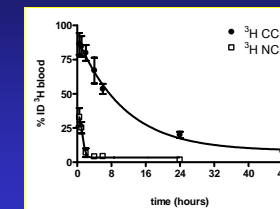
NCL vs CCL *in vivo*

- mPEG-*b*-p(20%HEMAMlac₁-co-80%HEMAMlac₂)
- 12% methacrylated
- CMT 12°
- ³H labeled
- M_w ~ 22000
- 14C tumour bearing mice

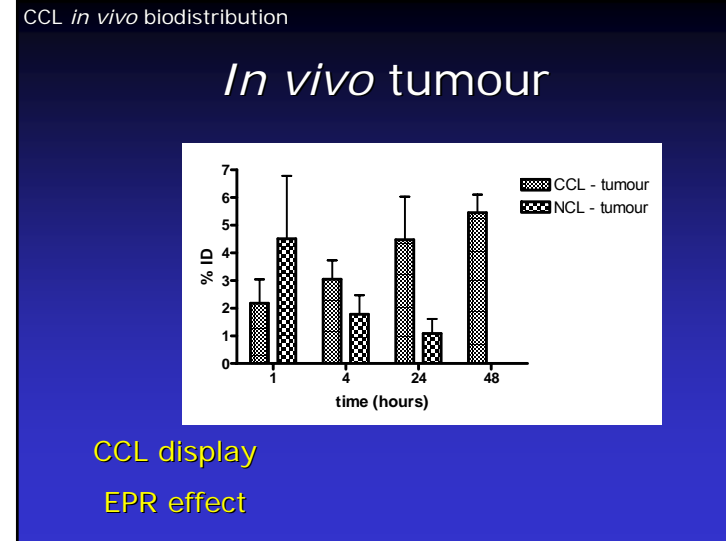
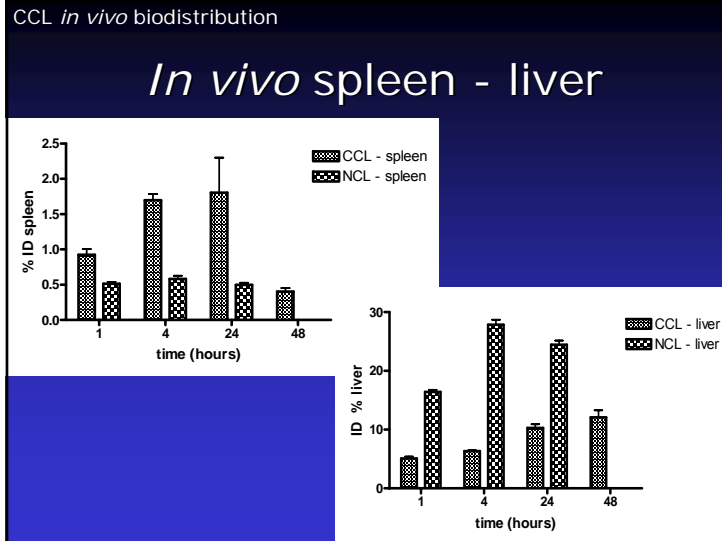
→ CCL and NCL micelles (57 nm)

In vivo circulation kinetics

Core-crosslinked (CCL) versus non-crosslinked (NCL) micelles



Chemical crosslinking of the micelles significantly prolonged circulation of the micelles



Conclusions

- Polymeric micelles can be used to solubilize PTX
- Excellent circulation kinetics of CCL micelles with retained/controllable biodegradability
- Promising biodistribution/tumour accumulation of CCL micelles *in vivo*

Acknowledgments



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