INNOWACYJNA GOSPODARKA
NARODOWA STRATEGIA SPÓJNOŚCI

IKiFP
im. J. Habera
PAN

UNIA EUROPEJSKA
EUROPEJSKI FUNDUSZ
ROZWOJU REGIONALNEGO

MULTIWARSTWY POLIELEKTROLITÓW W UKŁADACH DOSTARCZANIA LEKÓW

Krzysztof Szczepanowicz

Instytut Katalizy I Fizykochemii Powierzchni im Jerzego Habera,
Polska Akademia Nauk
Niezapominajek 8, 30-239 Kraków, Polska, ncszczep@cyf-kr.edu.pl

Projektu nr POIG.01.01.02-12-028/09 "Funkcjonalne nano i mikrocząstki - synteza oraz zastosowania w innowacyjnych materiałach i technologiach (FUNANO)"

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Współpracownicy
Karolina Podgórna,
Marta Łapczyńska,
Lilianna Szyk-Warszyńska,
Elżbieta Bielańska,
Marek Piotrowski,
Tomasz Kruk,
Piotr Warszyński

Sylwia Łukasiewicz
Monika Bzowska
Alicja Karabasz

Why do we need a pharmaceutical carrier?

Traditional pharmaceuticals rarely demonstrate specific affinity towards the site of their action and as a rule, they distribute throughout the body upon administration. To reach the action site, a pharmaceutical agent has to overcome the inactivating action of the aggressive biological medium and cross a variety of biological barriers, which frequently results in at least partial drug inactivation/degradation and unfavorable pharmacokinetics and biodistribution. In addition, many pharmaceutical agents could provoke multiple undesirable side effects in normal organs, tissues and cells. To solve these complicating issues, various systems for drug delivery are suggested.

Why do we need a pharmaceutical carrier?

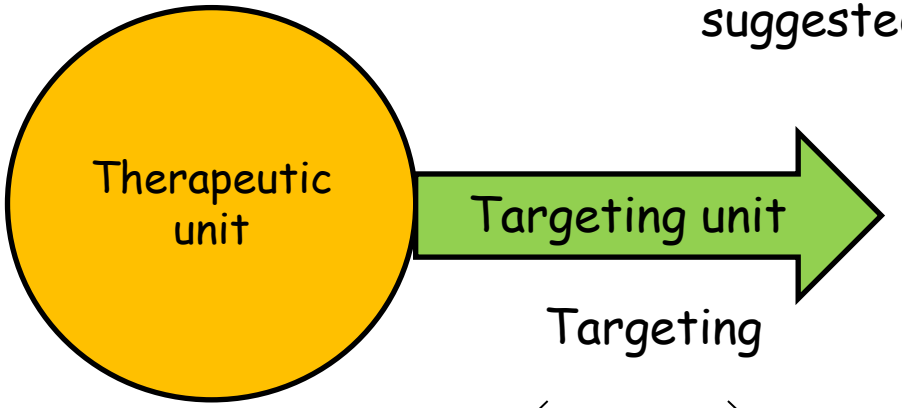
Protect a drug from the body
(which is especially true for unstable pharmaceuticals, such as protein and peptide drugs);

Protect the body from a drug
(which is especially true for highly toxic pharmaceuticals, such as chemotherapeutic drugs);

Favorably change drug pharmacokinetics
(which is especially required in cases when even stable and non-toxic drugs could be cleared from the body too rapidly to stably maintain their therapeutic concentration in the blood).

Concept of drug targeting

suggested by Paul Erlich



Drug
Drug carrier

Passive

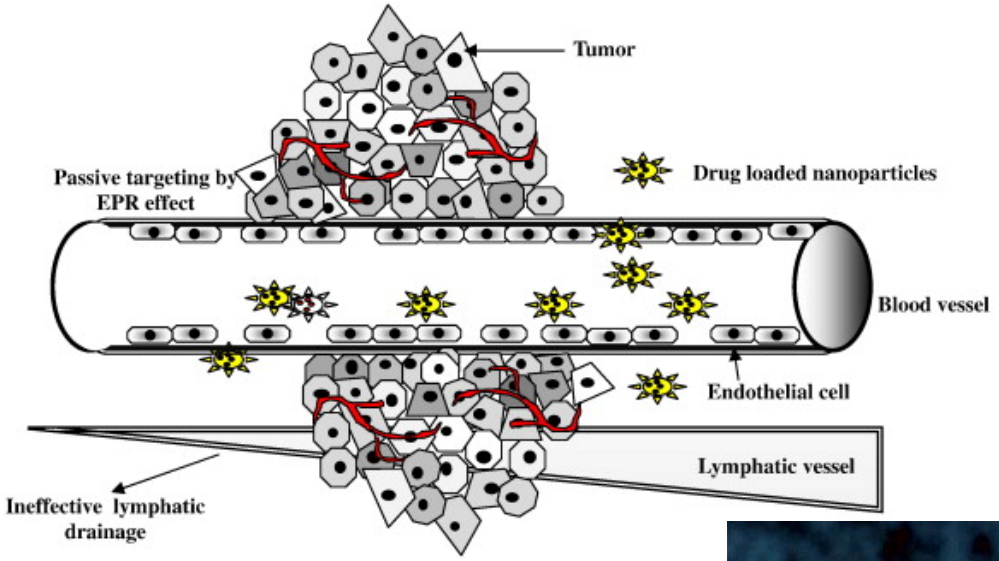
Liver,
Spleen,
Tumors (EPR)

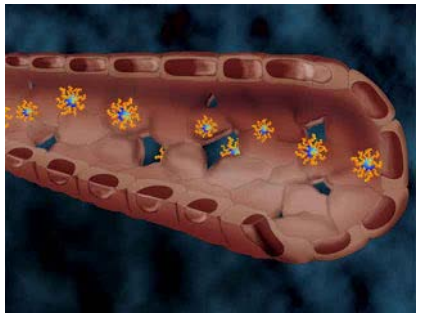
Active

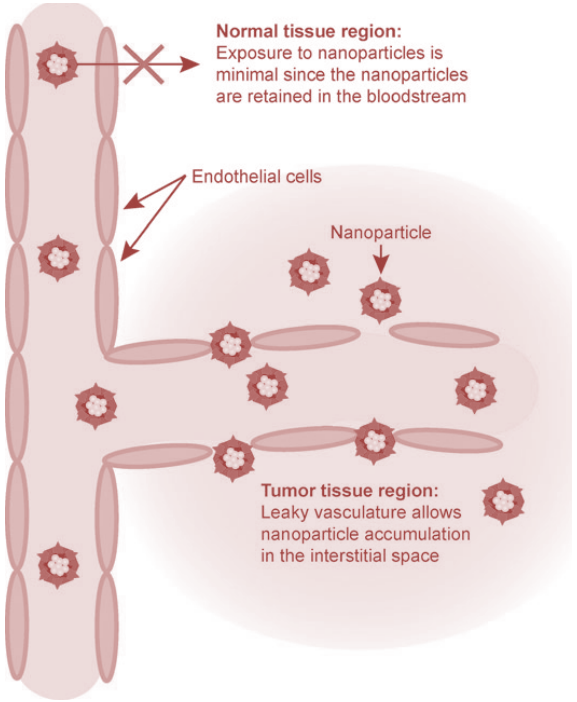
Targeting Ligands
Antibody, Vitamins,

Magnetic Drug Delivery

Passive Targeting:
EPR effect (Enhanced Permeability and Retention)

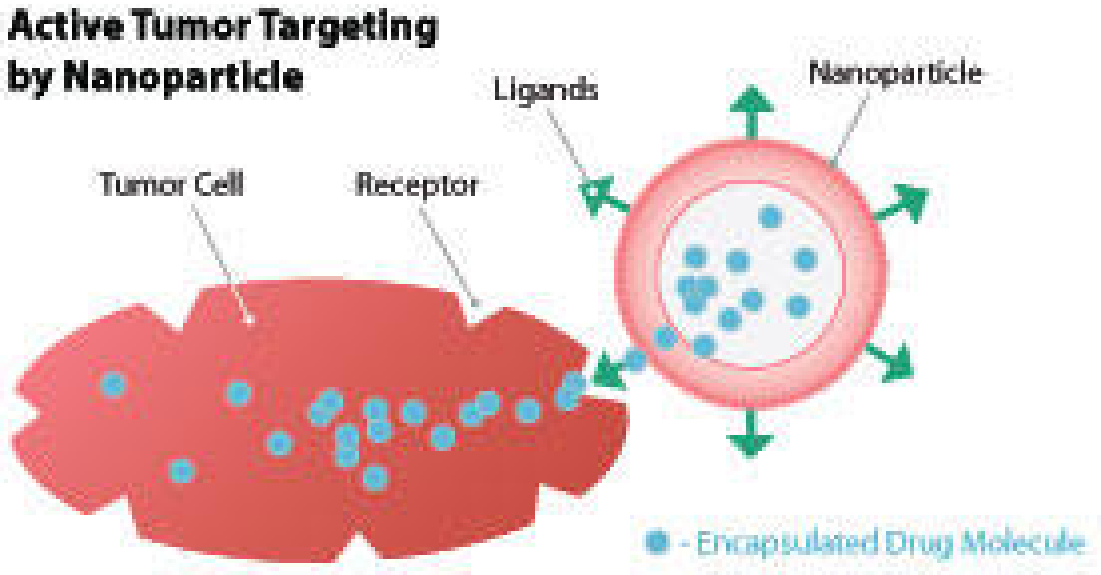






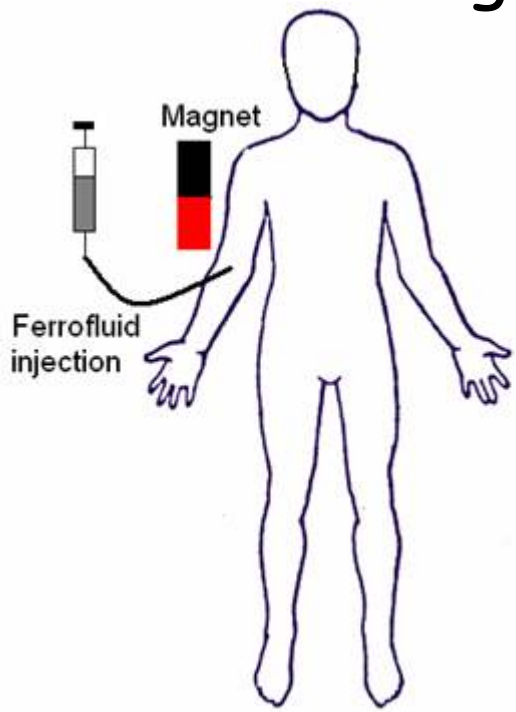
Active Targeting:

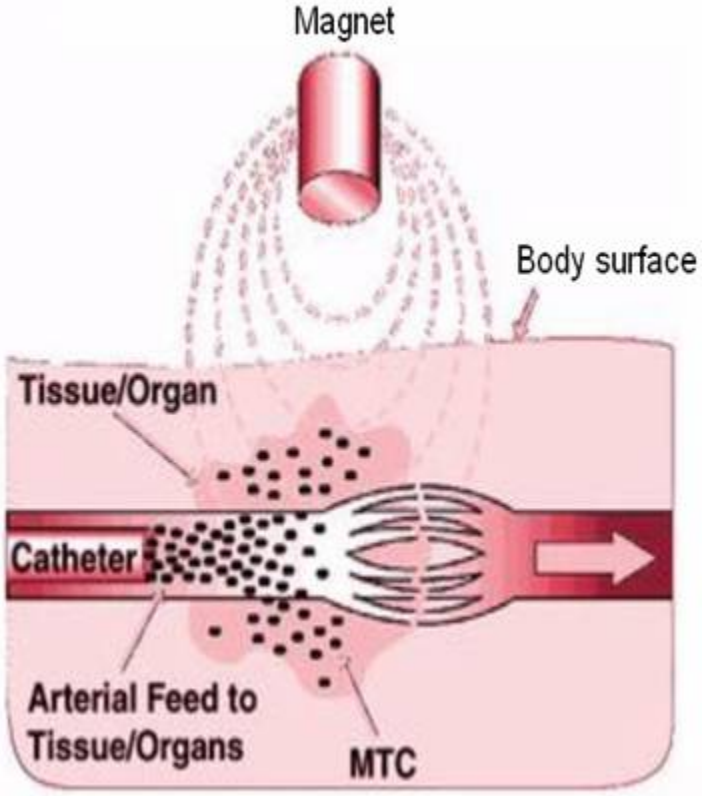
Targeting Ligands



http://www.understandingnano.com

Magnetic Drug Delivery





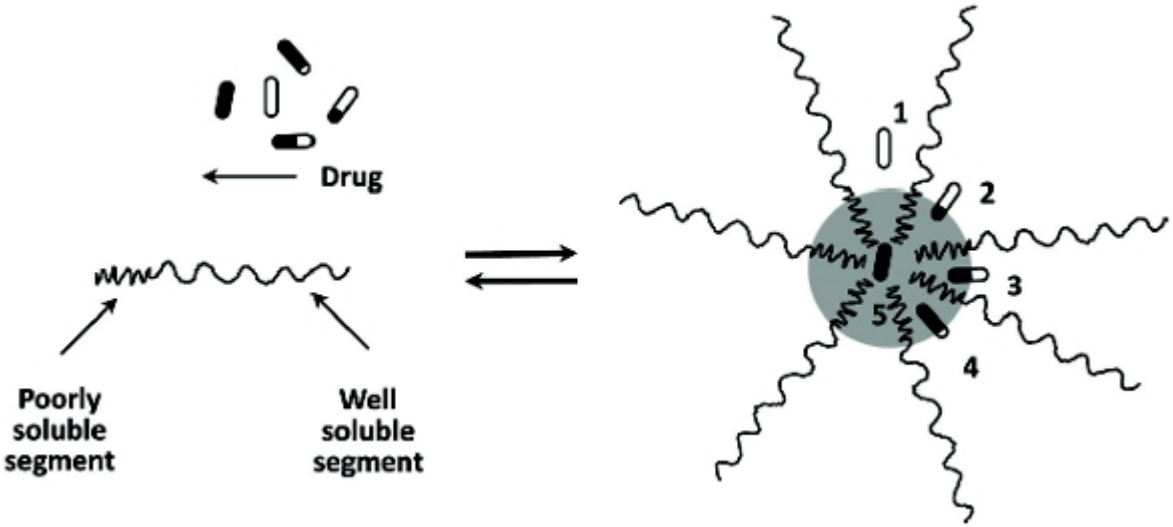
By applying a magnetic field gradient, it is possible to direct capsules to a region of interest. Due to the local accumulation of capsules, cells in this area can take up significantly more capsules than distant cells

Pharmaceutical nanocarriers of choice

An ideal pharmaceutical nanocarriers for intavenous adminstration

□Biocompatible & Biodegradable □Prolonged circulation
□Small size, high loading capacity □Multifunctionallity

Polymeric micelles

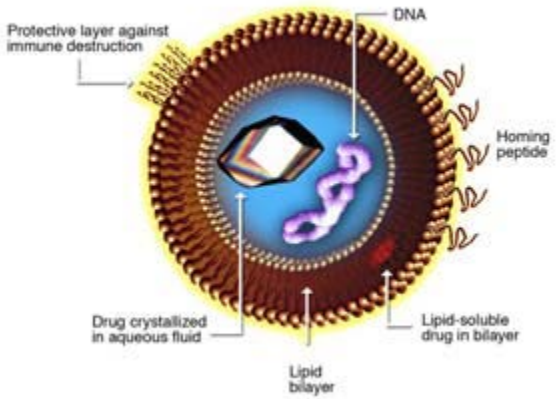


Pharmaceutical nanocarriers of choice

An ideal pharmaceutical nanocarriers for intavenous adminstration

□Biocompatible & Biodegradable □Prolonged circulation
□Small size, high loading capacity □Multifunctionallity

Liposomes

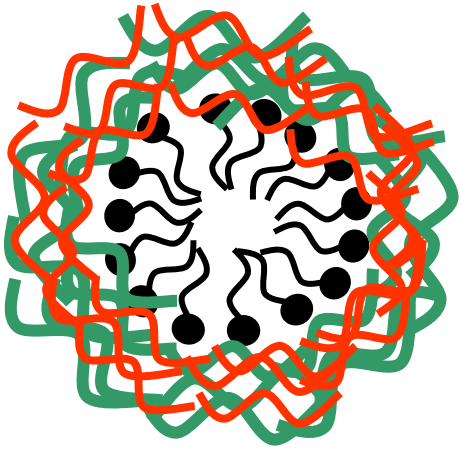


Pharmaceutical nanocarriers of choice

An ideal pharmaceutical nanocarriers for intavenous adminstration

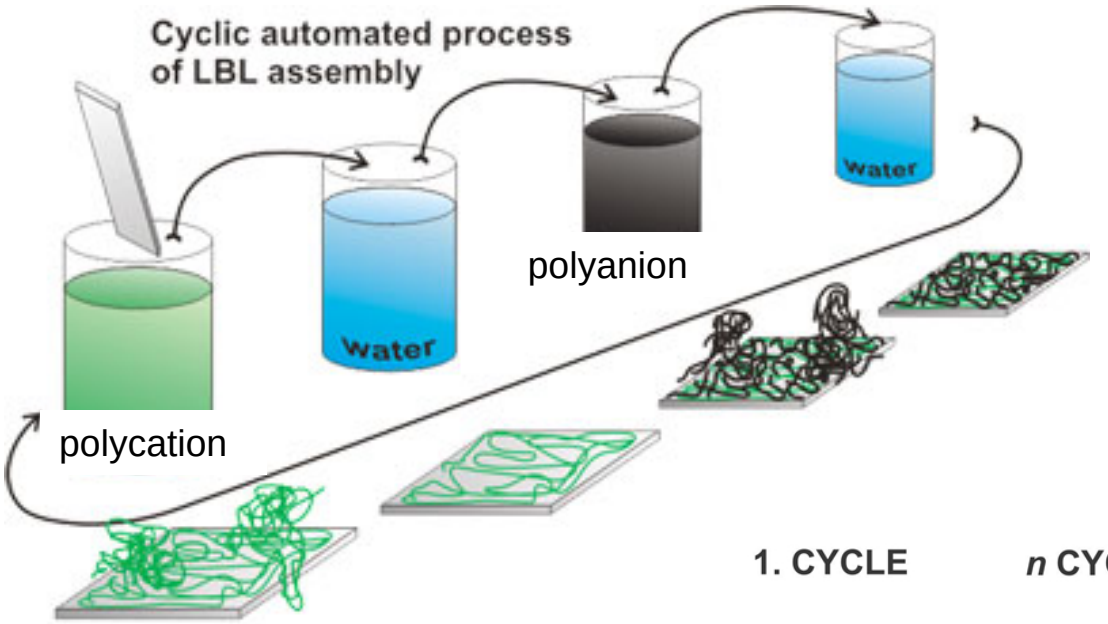
- Biocompatible & Biodegradable
- Prolonged circulation
- Small size, high loading capacity
- Multifunctionallity

Polyelectrolyte capsules

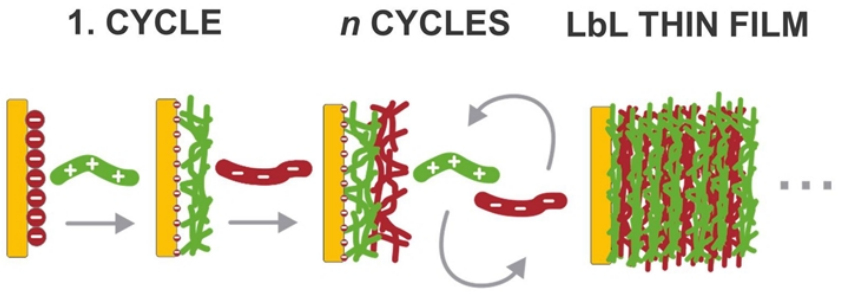


The main advantage of LbL capsules comes from the possibility of modification of the polyelectrolyte shell: by organic molecules, polymers, inorganic nanoparticles, carbon nanotubes, antibodies, by introduction of functional groups, lipids or nanoparticles. That multifunctionality can be utilized for preparation of targeted drug delivery systems.

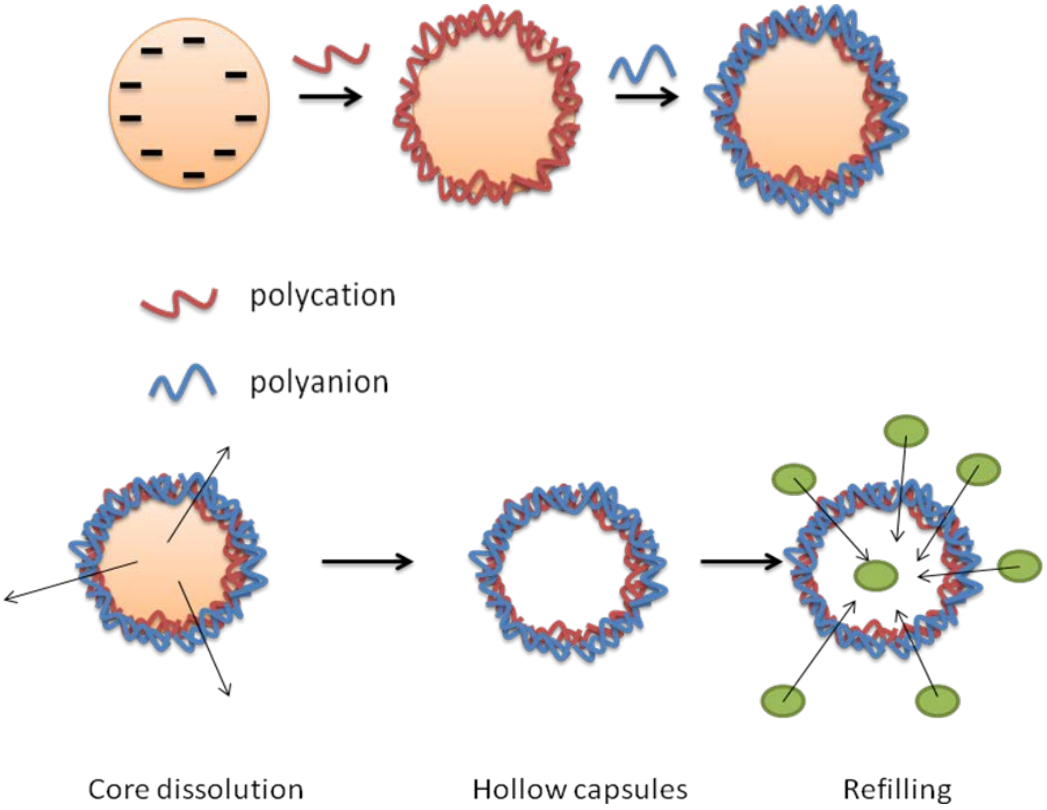
Polyelectrolyte multilayers- layer by layer



G. Decher



The layer-by-layer adsorption of polyelectrolytes (PE) is considered as a convenient method to obtain microcapsules' shells on colloidal cores



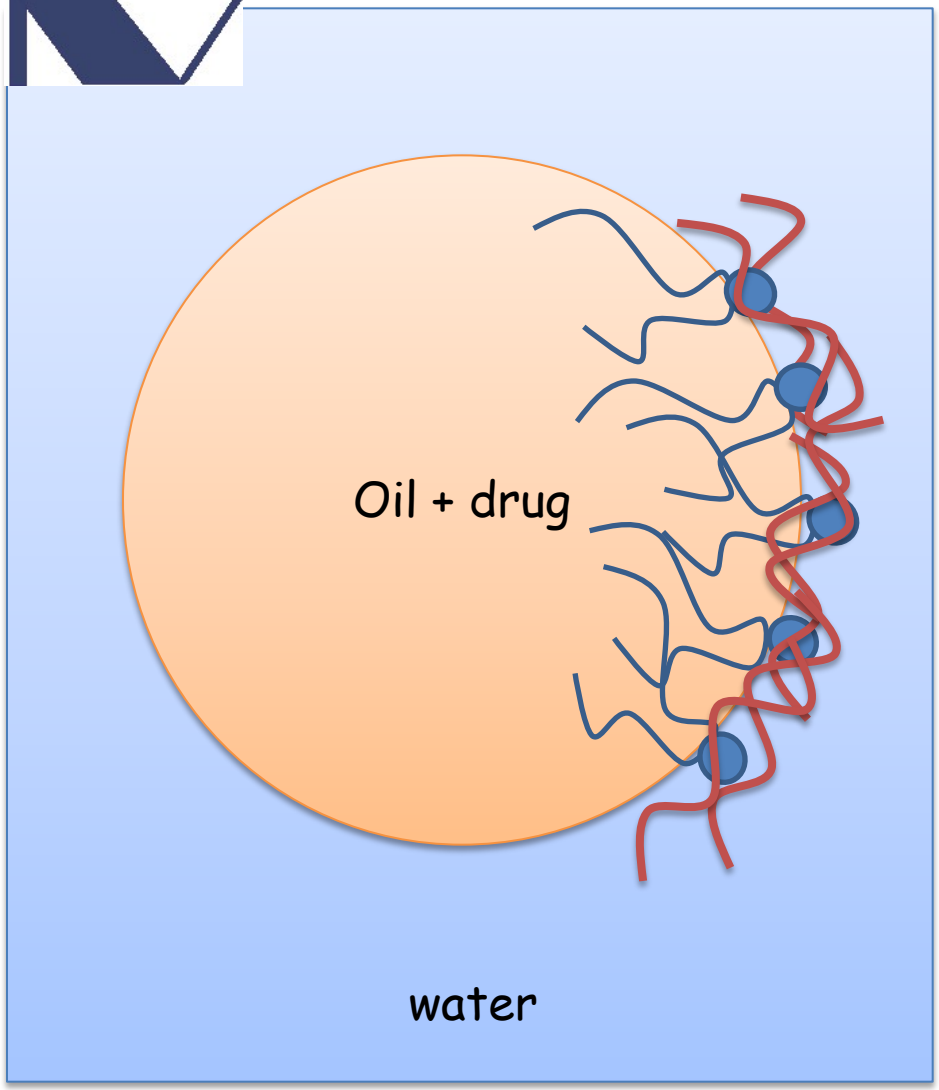
Solid particles (polystyrene latex, silica, $CaCO_3$) are most often used as cores for formation of capsules.

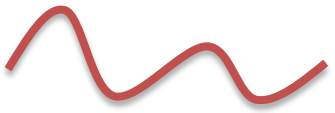
Disadvantages:
Core dissolution
low efficiency of loading

Sukhorukov, G. B.; Donath, E.; Lichtenfeld, H.; Knippel, H.; Knippel, M.; Budde, A.;Möhwald, H. *Colloids Surf. A* 1998, 137, 253-266

Our idea; use of emulsions droplets as liquid cores - give us possibility to encapsulate oil soluble active components with control of size and shell properties in one step process. (without core dissolution and refilling the shell)

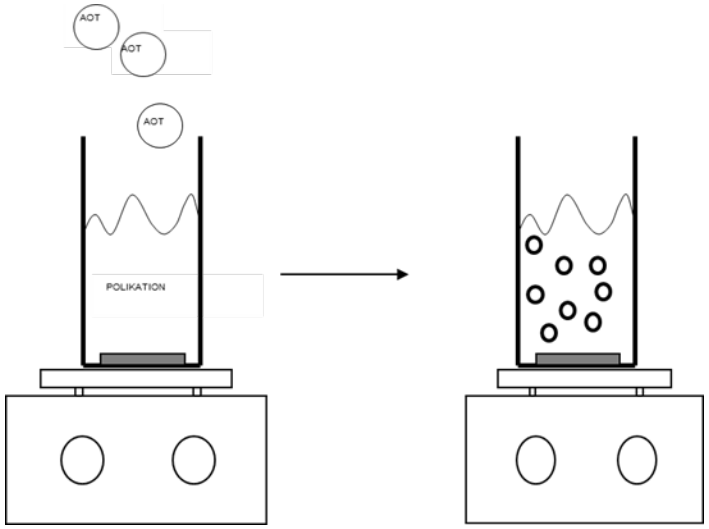
Nanocapsules preparation – Core preparation





Polycation

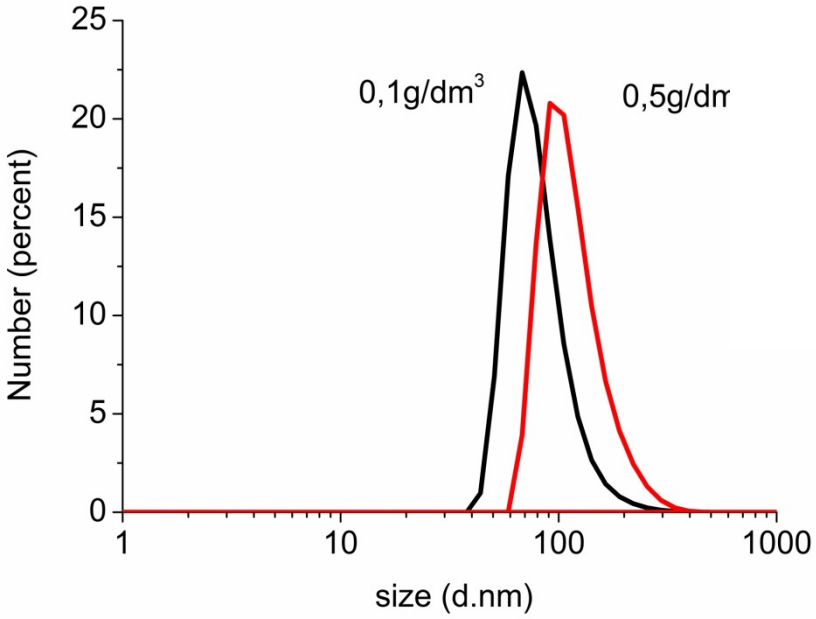
AOT- Aerosol OT – Docusate sodium salt

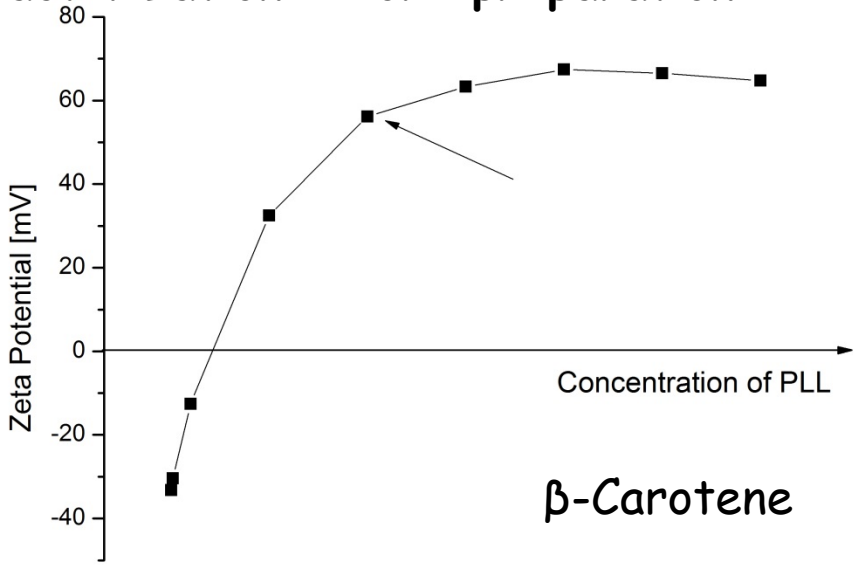


Szczepanowicz et al. , J. of Microencapsulation, 27(3) (2010) 198-204,

Capsules characterization – Core preparation

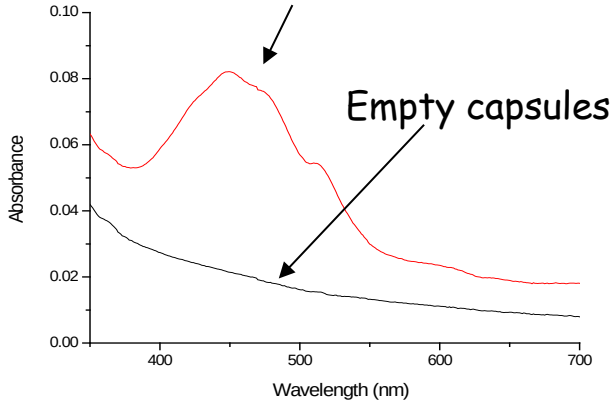
Size of capsules 60-200nm





β-Carotene

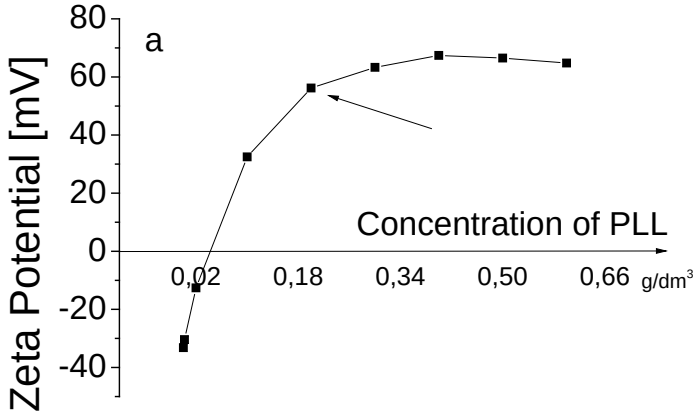
Capsules containing β-Carotene

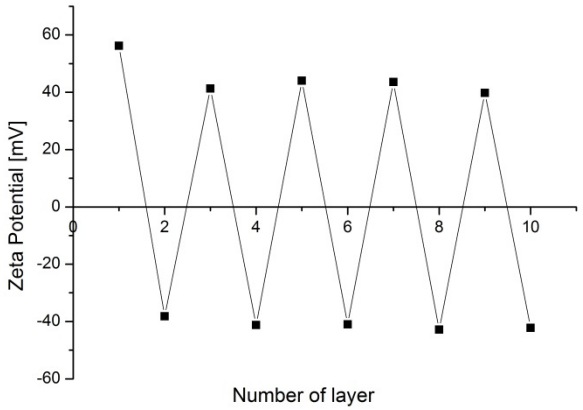


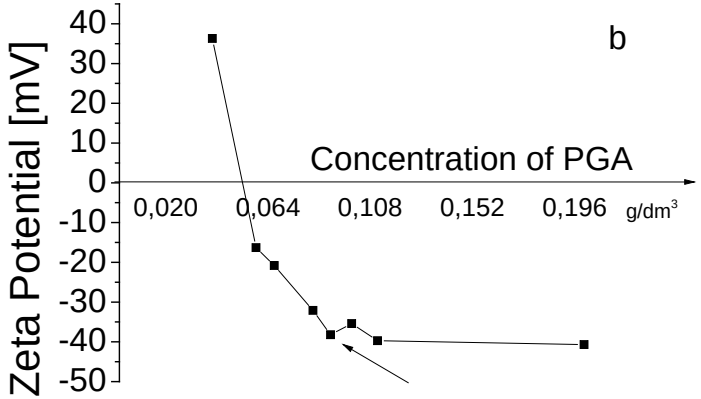
Polycations:
PLL
CHIT
PArg

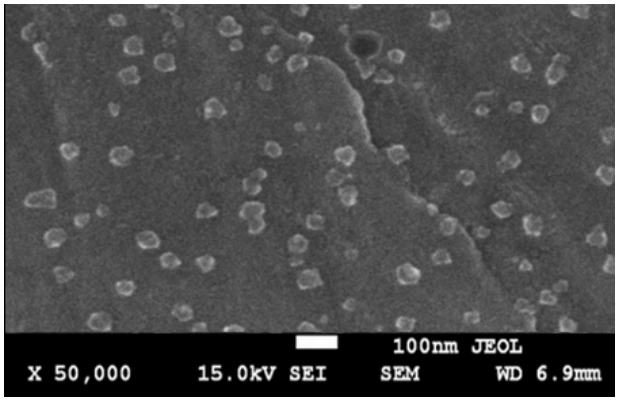
UV-VIS absorption spectra of empty capsules and capsules containing beta-carotene

Capsules characterization – shell formation







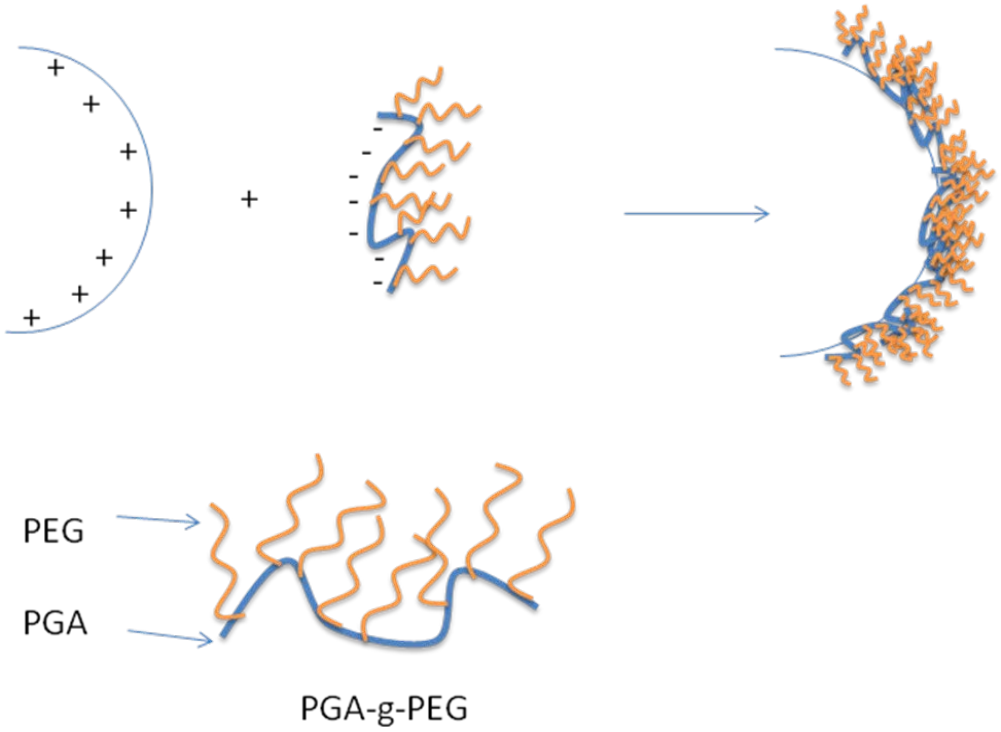


$AOT(PLL/PGA)_5$ –ten polyelectrolyte layer

Modification of shell/pegylation

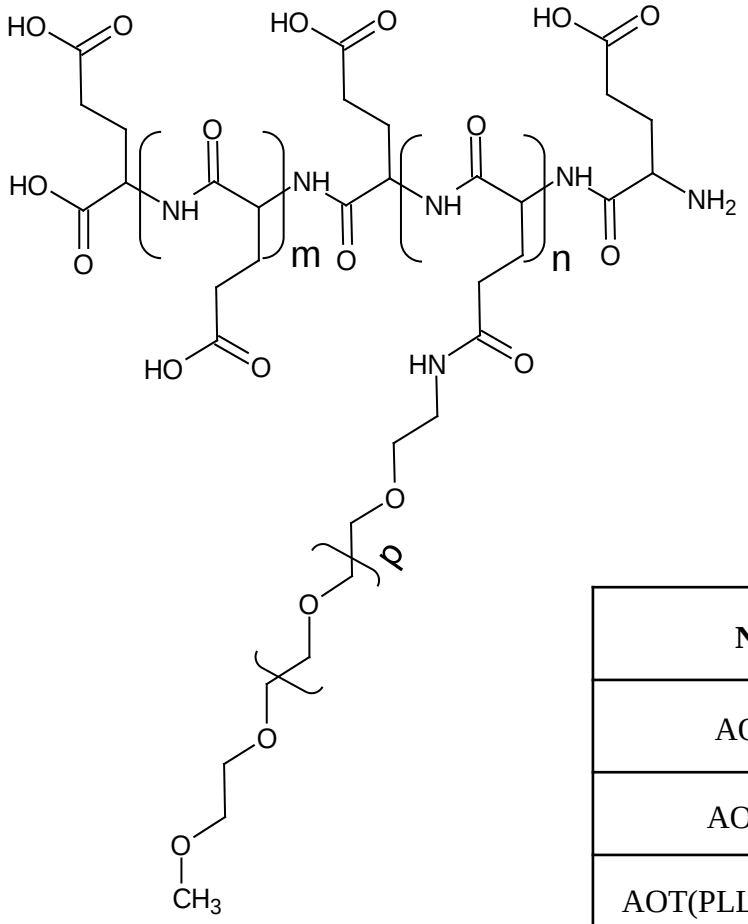
to prevent from nonspecific binding

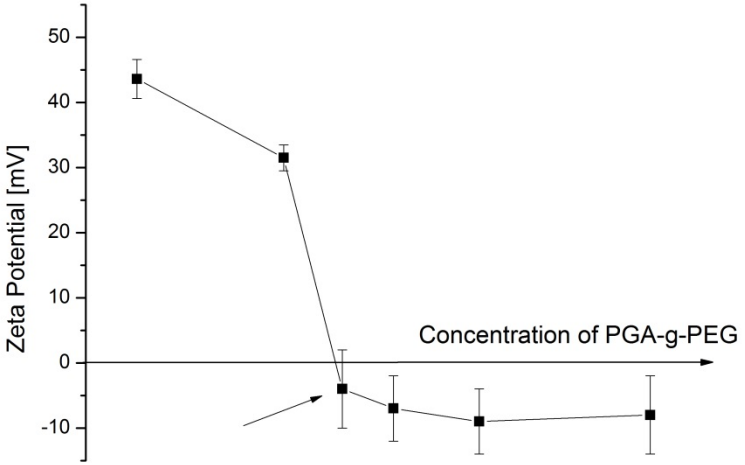
(elimination of opsonization and fast clearness)



To create pegylated nanocapsules nanocapsules were coated with PGA-*g*-PEG.

Modification of shell/pegylation



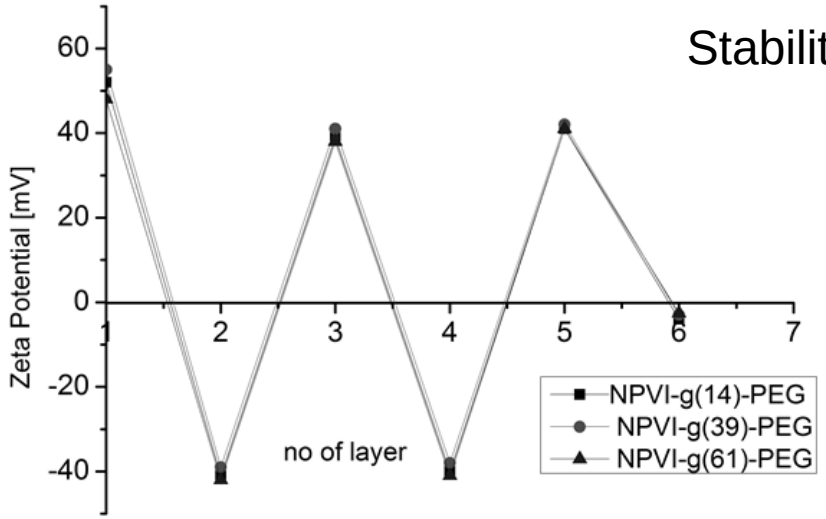


Nanocapsules	**Zeta potential [mv]**
$AOT(PLL/PGA)_3$	-41 ± 4
$AOT(PLL/PGA)_{3,5}$	39 ± 5
$AOT(PLL/PGA)_{3,5}$ PGA-g-PEG	-3 ± 4

PEG-1000, 2000, 5000
g-14%, 39%, 61%

Modification of shell/pegylation

A



B

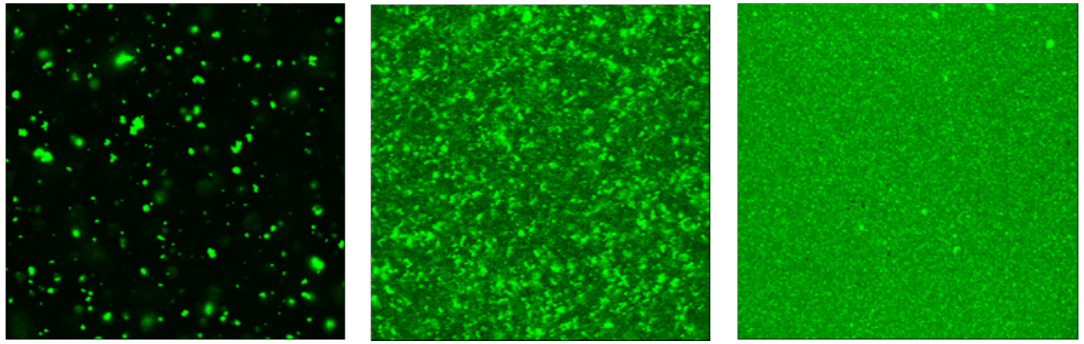
Stability in cell culture medium

PEG 5000

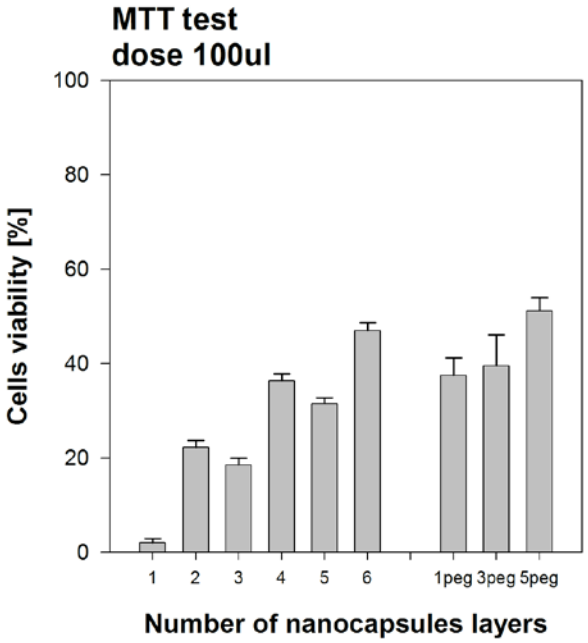
NPs V-PLL

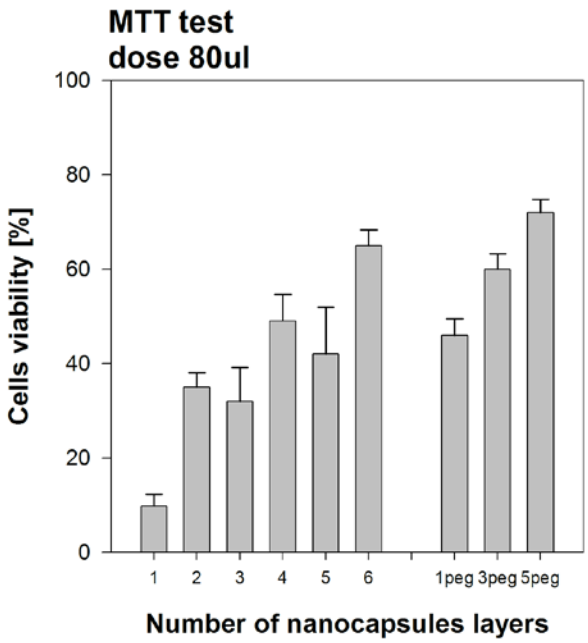
NPs VI-PGA

NPs VI
PGA-g(39)-PEG



Nanoparticles preparation. **A)** Dependence of NPs zeta potential on the adsorption of subsequent polyelectrolyte layers. **B)** Confocal microscopy analysis of NPs suspensions in cell culture medium

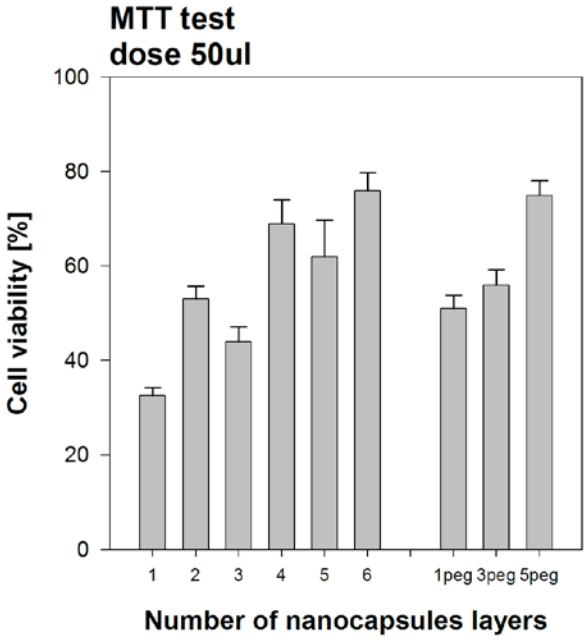


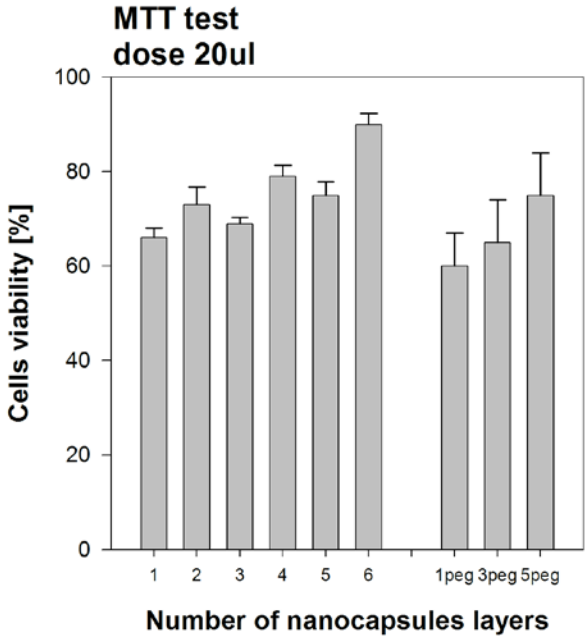


Cytotoxicity tests –
MTT, LDH, ATP

Depends on

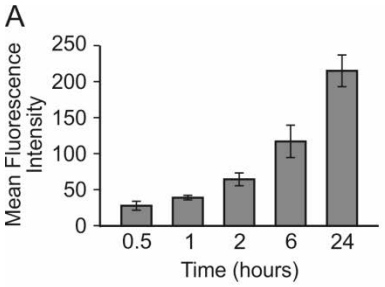
- Number of layer
- Dose
- Surfaace charge

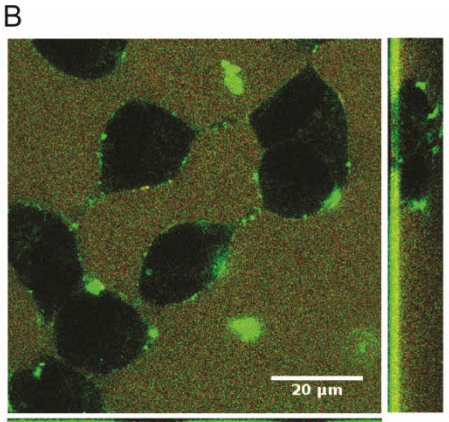




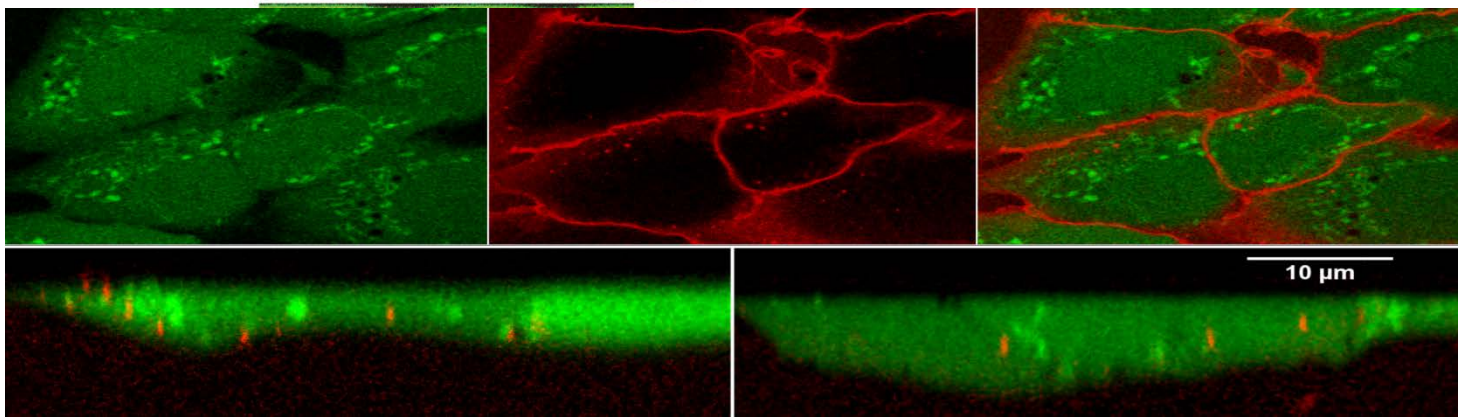
For the most toxic nanocapsules (with one polycation layer) about 90% cells could survive when the concentration of nanocapsules was $0,2*10^6$ per one cell

Cell penetration
Confocal microscopy studies



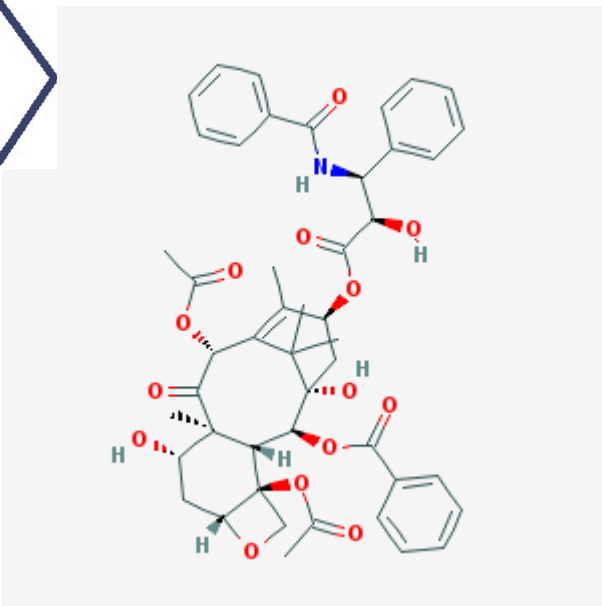


Analysis of NC uptake by *CT26-CEA cells. **A**,* Flow cytometry analysis performed after incubation of the cells for different periods of time with $NC2^{FITC}$ (10^{11}/ml).. **B**, Confocal microscopy image of CT26-CEA

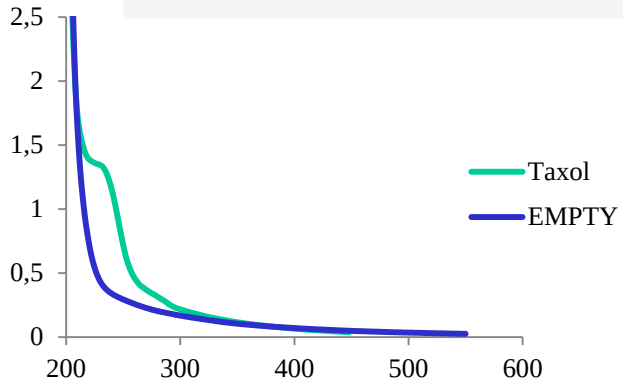


Horizontal and Vertical scan of HEK293 cell (green) after incubation with $AOT(PLL/PGA)_{3,5PGA}$-g-PEG (red).

Cancer treatment



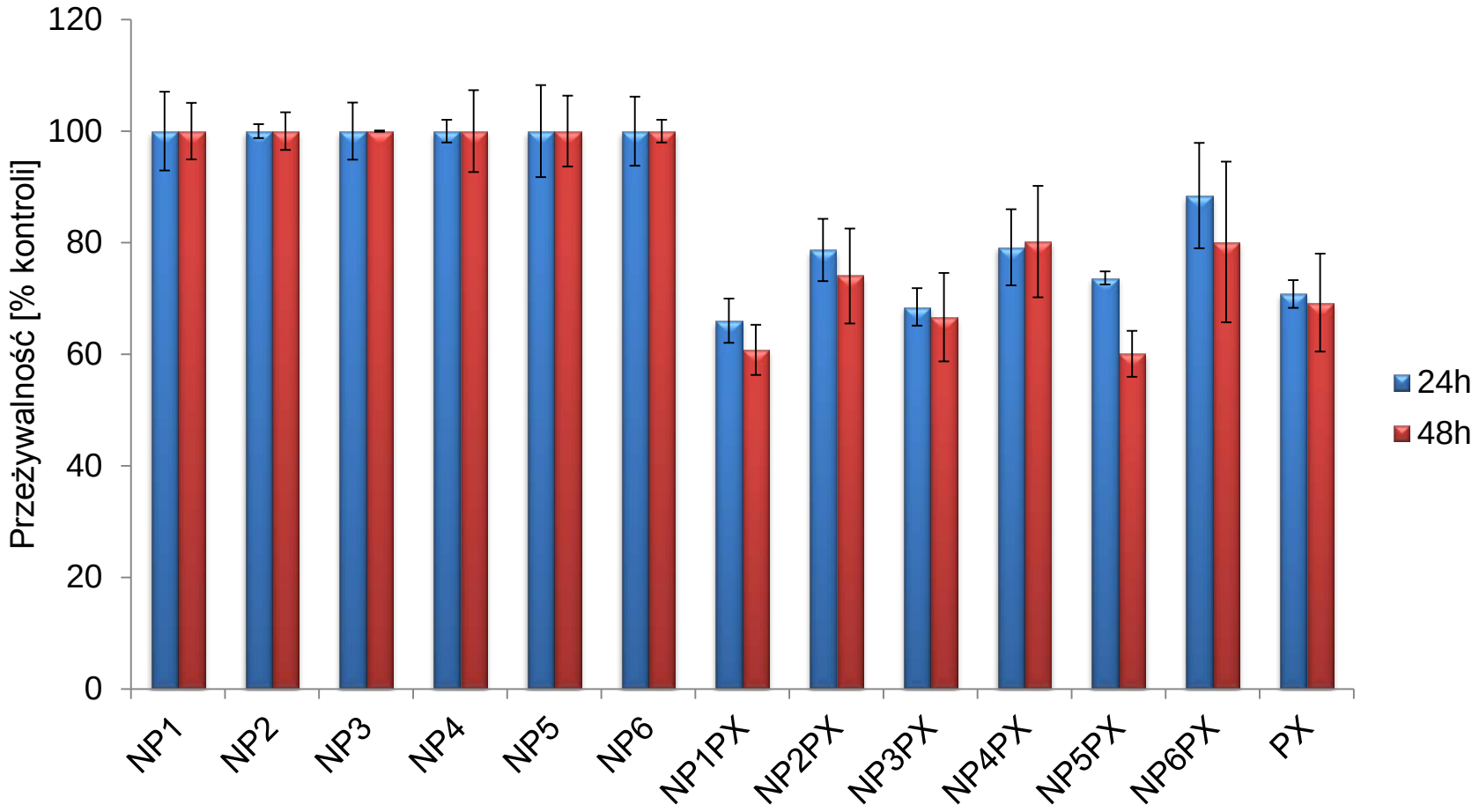
Paclitaxel is an anticancer agent isolated from the bark of the yew tree. It is classified as a microtubule-stabilizing agent and exerts cell killing effects by disrupting mitosis in dividing cells.



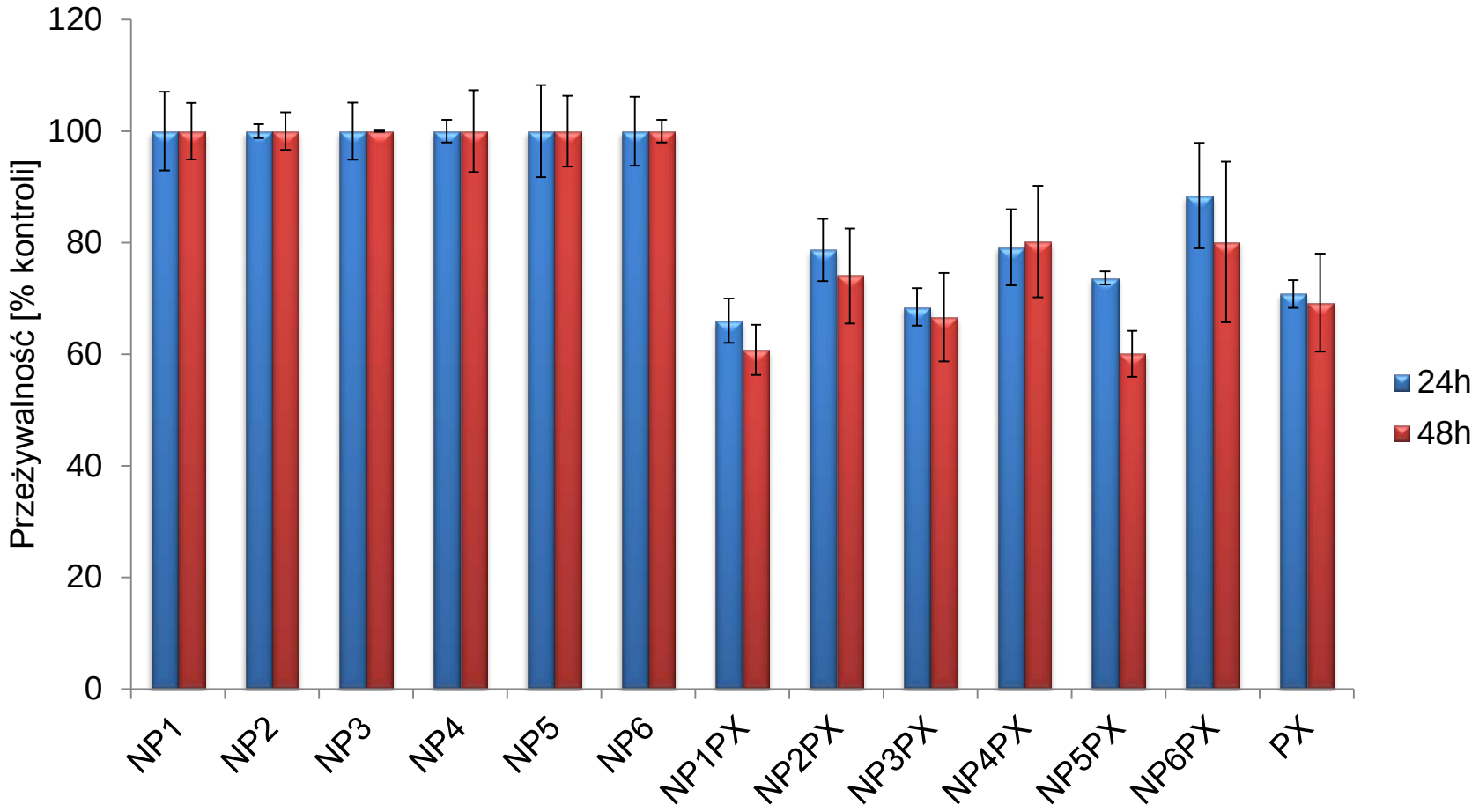
UV-VIS absorption spectra of empty capsules and capsules containing Taxol

Biodistribution < 6%

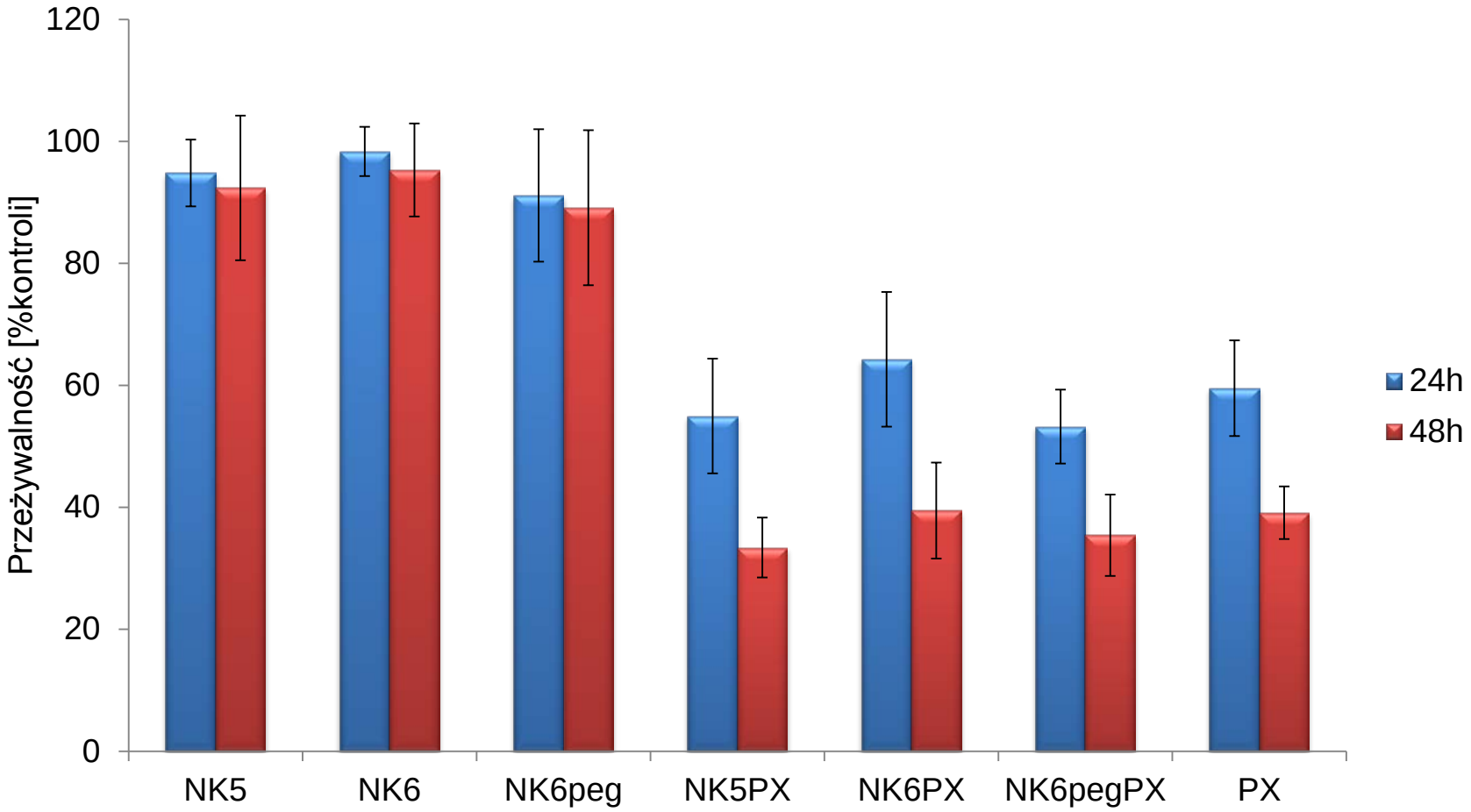
4T1, nanokapsulki multiwarstwowe



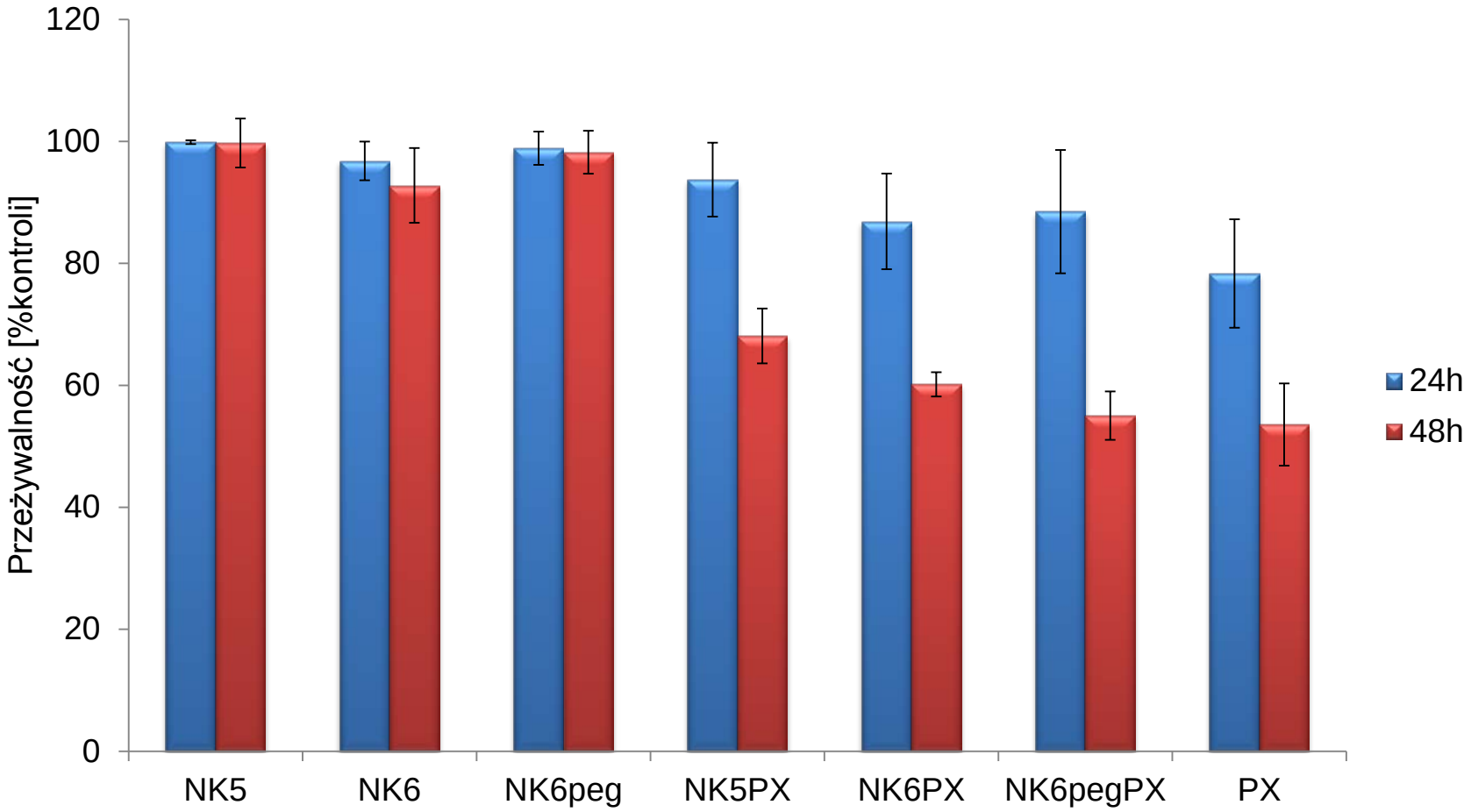
4T1, nanokapsulki multiwarstwowe



4T1 nanokapsułki PEGylowane (MTT)

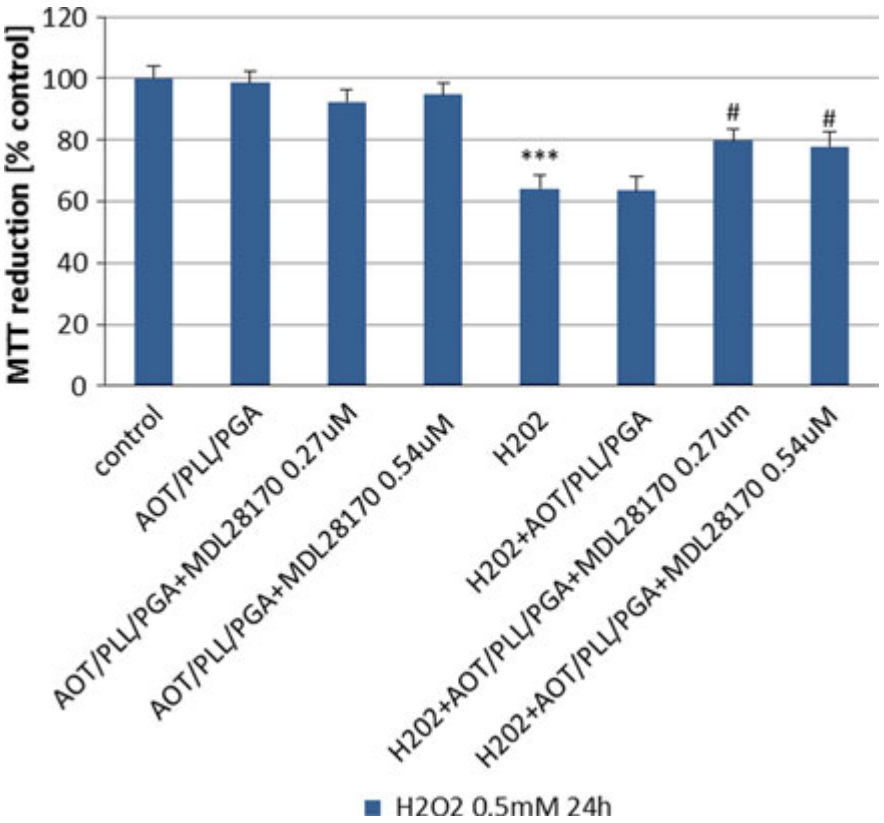


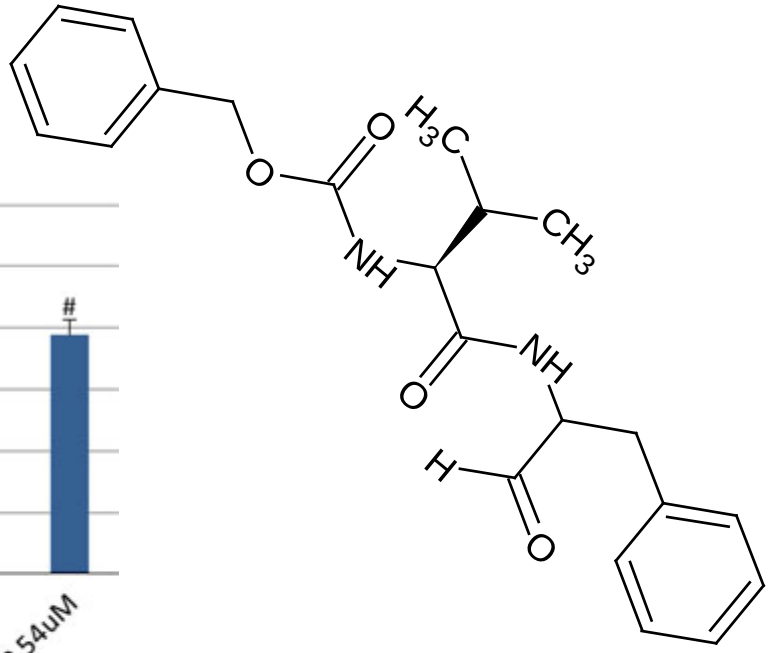
CT26CEA nanokapsułki PEGylowane (MTT)



Neurodegenerative disorders

MDL 28170

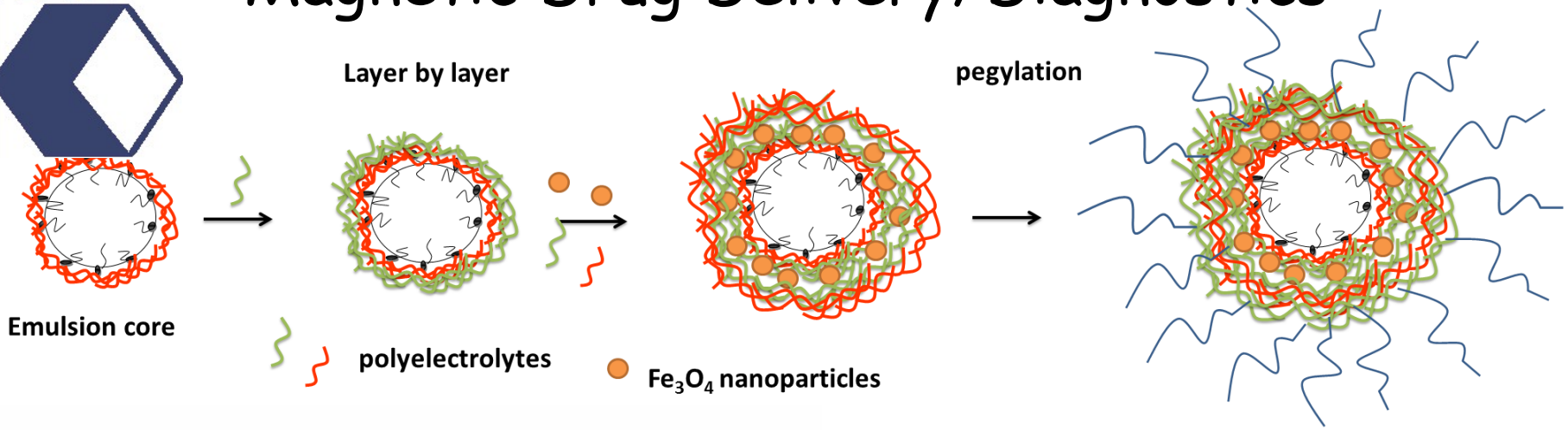


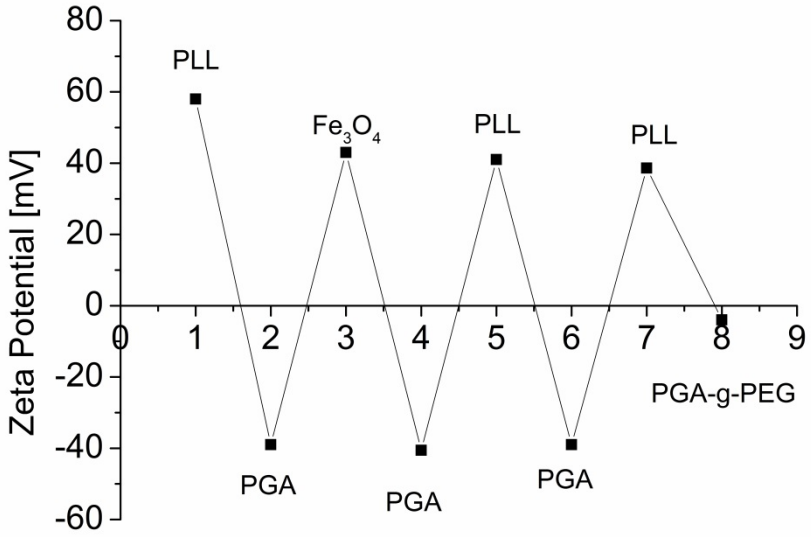


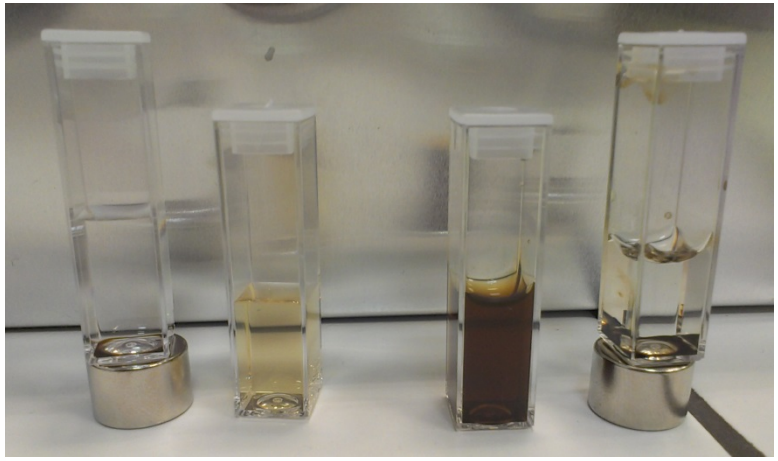
Neuroprotectants

The main advantage of nanocapsulated MDL 28170 is that it exhibits neuroprotective action at c.a. 40 times lower dose than the drug directly added to the assay.

Magnetic Drug Delivery/Diagnostics







Magnetic responsive nanocarriers/Nanocarriers of MRI contrast agent

Conclusions

- ✓ Nanocapsules with polyelectrolyte shells containing selected drugs were prepared
- ✓ Nanocapsules were modified for passive and active targeting
- ✓ Nanoencapsulated drugs show high activity
- ✓Our nanocapsules can be also used for diagnostics
- ✓ Our nano-scale drug carriers might serve as a novel, promising therapeutic agent for the targeted therapies

Thank you for attention !