Surface modification and colloidal stability of nano-carriers in physiological media

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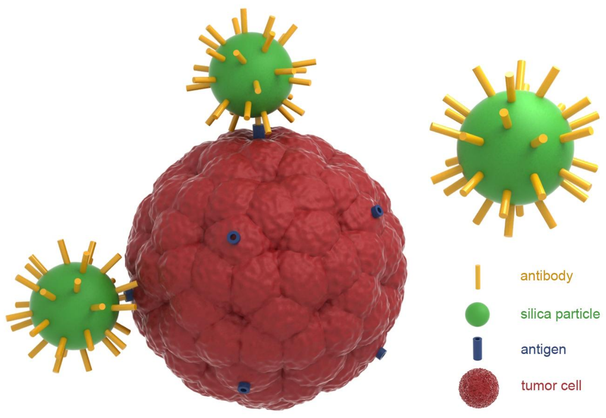
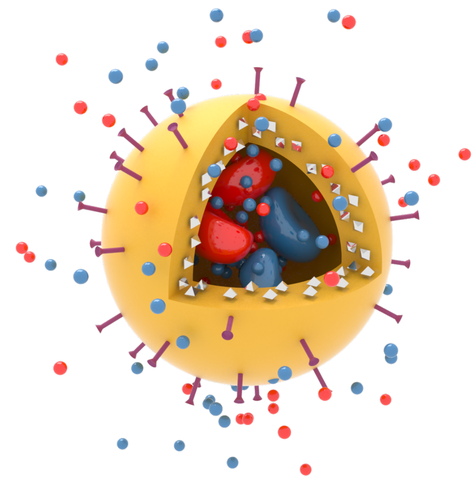
MOTIVATION



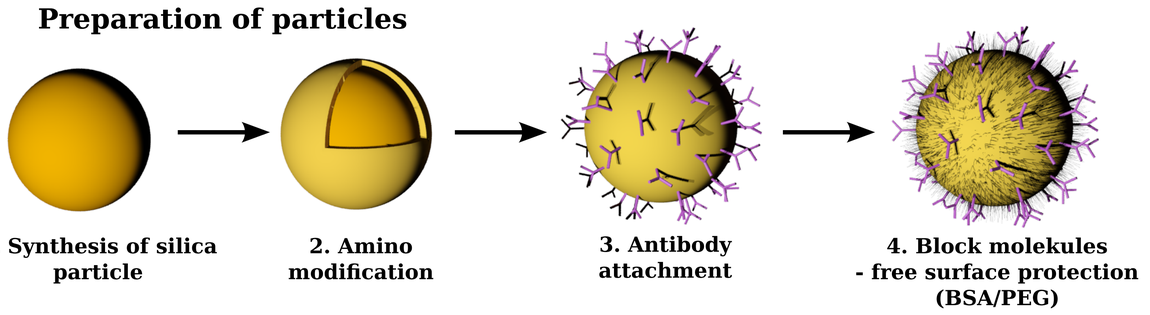
CHEMICAL ROBOTS

- complex structurated particel for effective drug delivery system
- smaller than erythrocyte (6-8 um in diameter)
- movement in blood circulation to find the target(s)
- antibody (AB) - antigen (AG) interaction
- nano/micro size
- accumulation of active substance inside the robot
- release cargo upon external stimuli (e.g. radiofrequency signal)
- utilization in pharmaceutical applications (e.g. treatment/diagnostic of cancer)
- iron nanoparticles for MRI visualisation
- IgG-M75 antibody specific for PG domain of carbonic anhydrase - expressed on colorectal carcinoma cell due to hypoxia



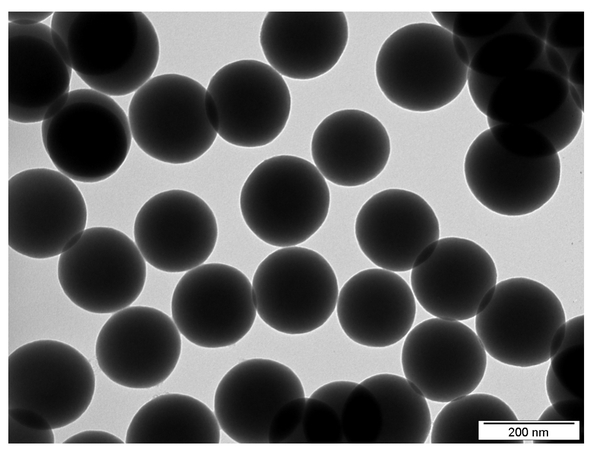
SILICA MODEL

Preparation of particles



1. Synthesis of silica particle
2. Amino modification
3. Antibody attachment
4. Block molekules - free surface protection (BSA/PEG)

- prepared by Ströber method
- 50-500 nm size in diameter
- fluorescently labelled (FITC)
- size measured: TEM/SEM and DLS
- various surface modification (amino or silanized in our case)
- coupling of IgG-M75 antibody or conrol antibody IgG-X
- functionality confirmed by ELISA-like test or flow cytometry test (FACS)
- BSA or PEG used for blocking free surface on particle

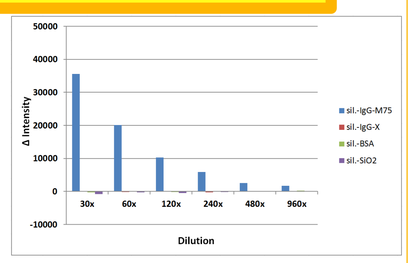


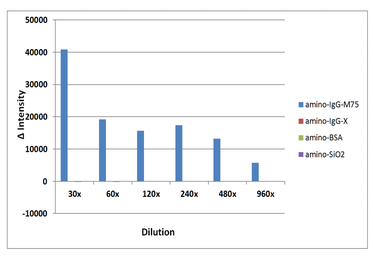
TEM image of SiO_2 particles

SILANIZED PARTICLES

ELISA-like test

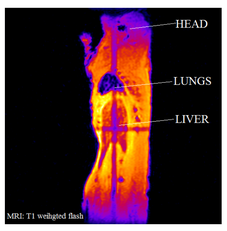
- PG-MBP antigen
- different dilution of particles
- strong adhesion between specific antibody and antigen PG-CA IX in comparison to controls

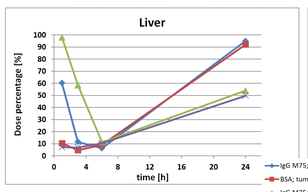


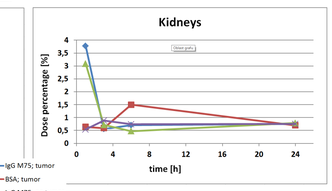


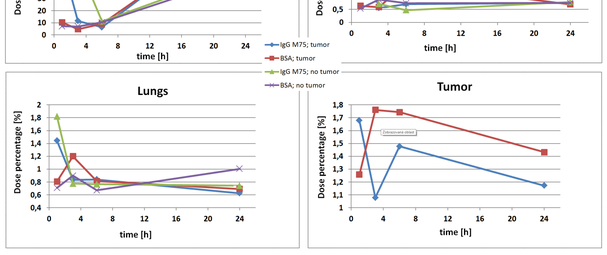
In vivo experiments

- Nu/Nu mouse
- determining of biodistribution pacticles according to surface modification
- measured fluorescence in homogenized tissue
- MRI visualisation





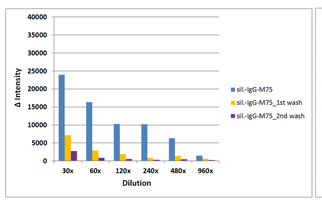


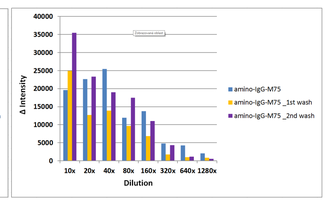


AMINO - PARTICLES

Covalent bond

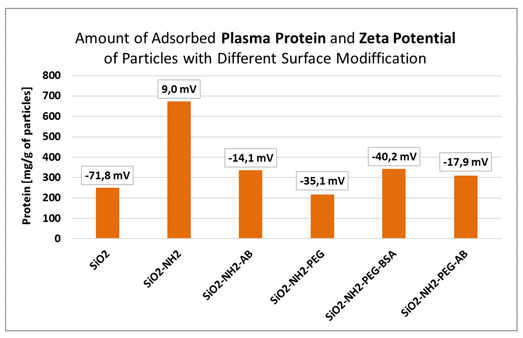
- antibody is adsorbed on silanized particles and it is wash by PBS or other buffers
- on the amino-particles the antibody is attached by covalent bound





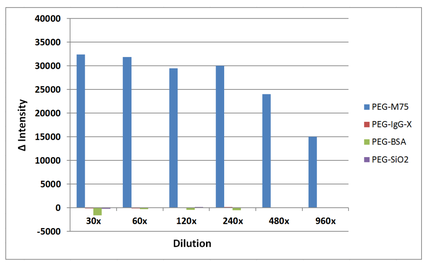
Plasma proteins

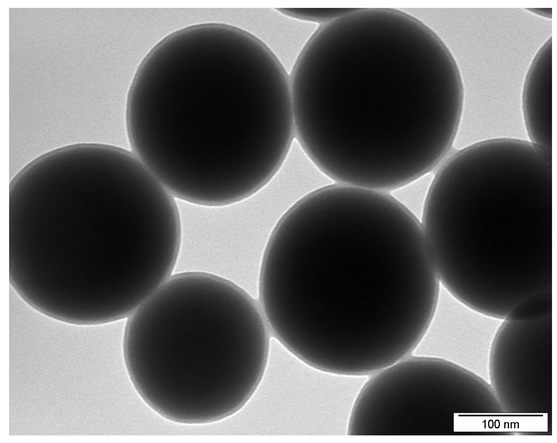
- binding of plasma protieins on particle is undesirable
- activation of macrophages
- blocking the active side of antibody
- zeta-potential also affects amount of adsorbed plasma proteins



PEG particles

- polyethylenglycol chains
- do not affect AB-AG interaction
- pegylation prevents adsorption of plasma proteins-movement in liquid medium

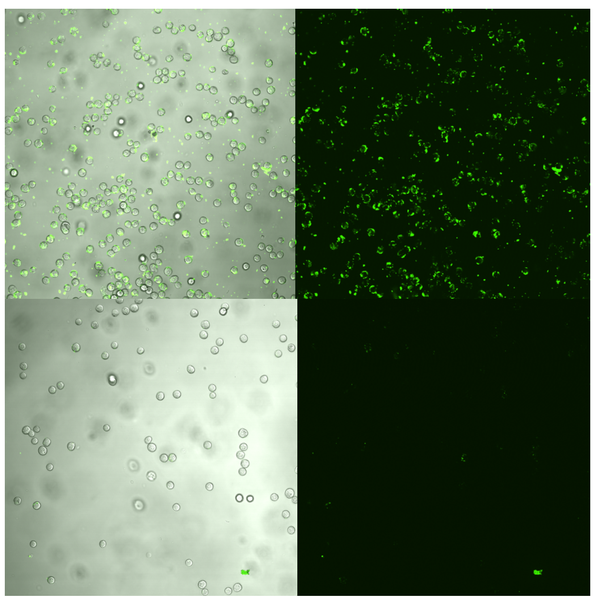




SiO_2 particles with amino layer

Adhesion tests

- HT-29 cell - expressed carbonic anhydrase on its surface
- 30 minutes incubatin with particles
- confocal microscopy



CONCLUSION

- Silica particles with covalent bound of IgG-M75 exhibit high specificity for PG.domain of CA IX in comparison to particles with BSA/IgG-X modification.
- Surface modification with PEG chains decreases the amount of plasma proteins interacting with the particle surface and allows specific interaction between IgG-M75 and PG-CA IX.

Future perspective

- test amino-silica nanoparticles modified by IgG-M75 in fluid 3D cell model
- optimalize amount of PEG attached to amino-silica particles
- use another structure for antibody attachment
- test amino-silica nanoparticles modified by IgG-M75 in vivo model (Nude mouse)

References: Tokárová V., Pittermannová A., Král V., Řezáčová P., Štěpánek F., “Feasibility and constraints of particle targeting using antigen-antibody interaction” Nanoscale 5, 11490-11498 (2013).
Závada J., Závadová Z., Pastorek J., Biesová Z., Ježek J., Velek J., “Human tumour-associated cell adhesion protein MN/CA IX: identification of M75 epitope and of the region mediating cell adhesion” British Journal of Cancer 82(11), 1808-1813 (2000).

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