Appropriate way of antibody coupling to SiO_2 nanoparticles

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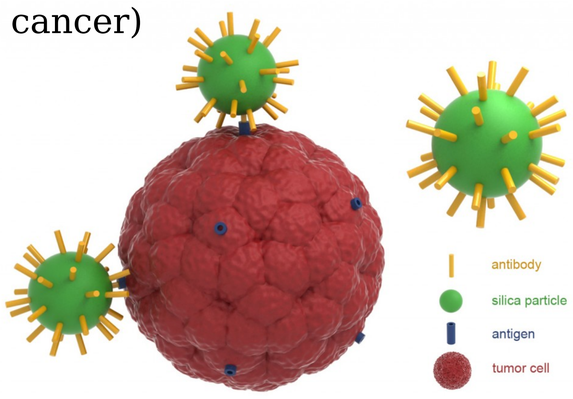
Introduction

Chemical robots

- Composite nano-/micro-particles
- Accumulation of active substance inside and release it on the demand
- Utilization in pharmaceutical applications (e.g. treamtment of cancer)



Aims of Work

- Surface modification of chemical robots which can allow the target delivery of drugs
- Optimalization the way of antibody coupling on a particle surface with respect to coverage density, specificity and adhesion effect to corresponding antigen or cancer cells

Methodology

- Nano-silica particles are used as a model for covalent surface modification by monoclonal IgG-M75 antibody
- IgG-M75 antibody binds the PG domain of carbonic anhydrase (PG-CA IX) which is highly expressed in tumor cells due to hypoxia
- Silica nanoparticles were modified by three types of protein: specific antibody IgG-M75, unspecific antibody IgG-X (IgG from human serum) and protein structure BSA (bovine serum albumin)
- Adhesion ability of modified nanoparticles was examined with cells HT-29 (derived from human colorectal carcinoma) which express PG-CA IX and human DLD1 (do not express the PG-CA IX).

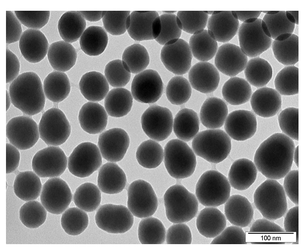
Methods

- Silica nanoparticles are synthetized according to the Stöber process with addition of fluorescein isothiocyanate
- Characterization by the dynamic light scattering method (size analysis) and by the transmission electron microscopy (size and morphology)
-Successful coupling of IgG-M75 antibody was confirmed by the ELISA-like and flow cytometry tests with specific antigen domain (PG domain of carbonic anhydrase) and cancer cells
- Adhesion tests were performed with HT-29 and DLD1 cells analysed by confocal microscopy

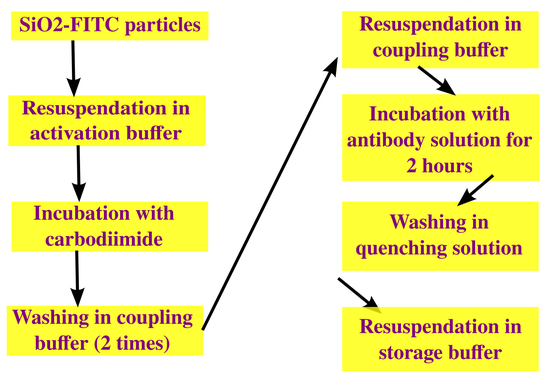
Results

Silica particles

- Size range: 50-100nm
- Spherical morphology
- Fluorescent labelled

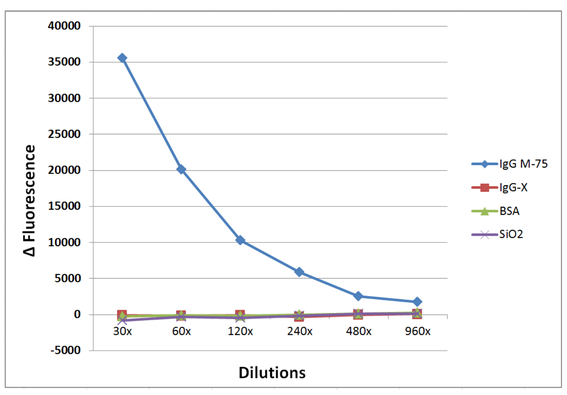


Coupling of antibody

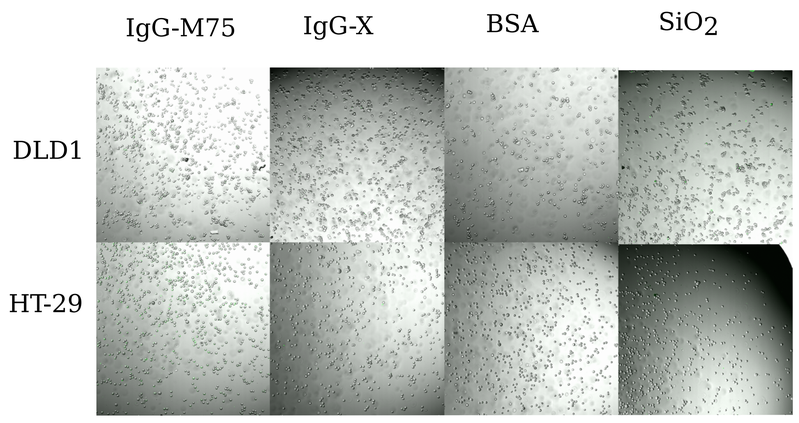


ELISA-like test

- PG-MBP antigen
- Silica particles coupling with IgG-M75, IgG-X and BSA
- Negative controls IgG-X and BSA prepared in the same way like as antibody IgG-M75
- Different dilution of particles
- Strong adhesion between specific antibody IgG-M75 and antigen PG-CA IX in comparison to unspecific antibody IgG-X, protein BSA and unmodified SiO2 particles



Adhesion test



- HT-29 and DLD1 cells
- Negative controls IgG-X and BSA prepared in the same way like as antibody IgG-M75
- Cells incubated with accutase solution for 15-20 min
- Particles diluted 50x
- Confirmation of strong adhesion of IgG-M75 modified particles compared to other particles

Conclusion and Future

Conclusion

Silica nanoparticles modified by specific antibody IgG-M75 are able specifically to bind to the HT-29 cells compared to particles modified by unspecific IgG-X and protein structure BSA or unmodified particles

Future perspective

- Create a coverage/density model of silica nanoparticles
- Find out the kinetics of IgG-M75 coupling to silica nanoparticles and the strength of antibody-antigen bound
- These findings approach in the case of another particles, such as liposomes etc.
- Adhesion and fluid tests in 3D

Reference 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).

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