Fluorescent nanoparticles-based new technology: preliminary studies for future applications as diagnostic tool and drug carrier

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Background

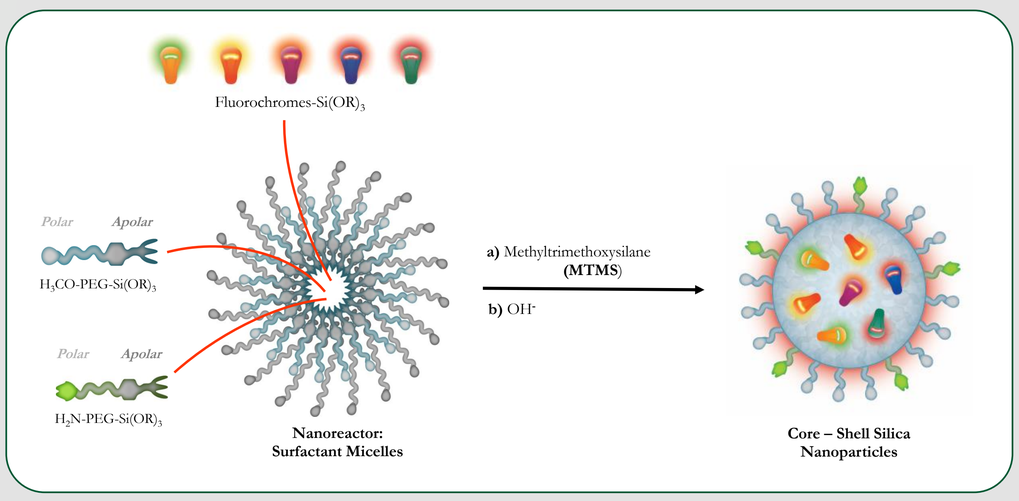
Since most biologically active macromolecules are natural nanostructures, operating in the same scale of biomolecules gives the great advantage to enhance the interaction with cell membrane and cellular proteins. Thanks to their unique features of shape, size and charge, nanoparticles (NPs) appeared to be good candidates in a wide range of applications. This extremely versatile technology may also offer the possibility to combine multiple features providing, for instance, imaging and therapy in the same construct. Multifunctional nanoparticles have shown great promise in emerging medical fields such as multimodal imaging, theranostics and image-guided therapies. AcZon NPs are fluorescent core-shell silica nanoparticles emerging as promising probes able to overcome some limits of traditional fluorochromes as lack in stability and low intensity emission in water solution. Concerning fluorescence, silica has proven to be an excellent tool: it is photophysically inert, not involved in energy or electron transfer processes and, moreover, intrinsically non-toxic. The most innovative feature of AcZon NPs is the ability to be a platform where the fluorescence energy transfer process, known as FRET, occurs at a high efficiency rate. Besides being the most used "stealth" polymer in the drug delivery field, polyethylene glycol (PEG), which composes NPs shell, allows to modulate the type and number of functional groups (e.g. amine, thiol, carboxyl or methacrylate) exposed on NPs surface. As a consequence, PEG properties lead to the conjugation of several biomolecules on AcZon NPs. Amine reactive groups, for instance, can be linked to monoclonal antibodies via crosslinkers, through a site-specific conjugation, preserving antibodies biological activity.

Aim

The aim of the present study is the investigation of AcZon NPs uptake in a cellular model, U937 (human histiocytic lymphoma cell line) and the Ab-NPs conjugated targeting capability on whole peripheral blood, as preliminary studies for future applications.

AcZon technology

AcZon NPs are synthesized through a micelle-assisted method, where a surfactant is used to create a nanoreactor within which reagents arrange. The base-catalysed hydrolysis of different trialkoxysilanes leads to the formation of monodisperse NPs.



Shell is composed by two different polyethylene glycols (PEG), both terminating with a trialkoxysilane group:

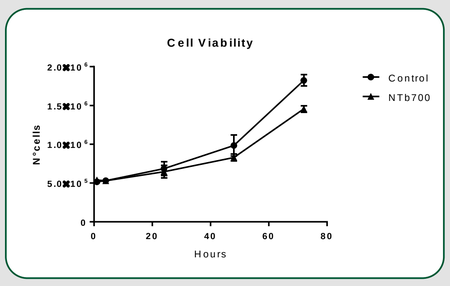
- H_3CO-PEG-$Si(OR)_3$: main component of the shell. Induces stability and solubility in water;
- H_2N-PEG-$Si(OR)_3$: allows the presence of amine reactive groups on the external shell.

To obtain nanoparticles excitable with blue laser and emitting in the near-IR (**NT_B700**), five different dyes were selected to be simultaneously entrapped into silica core. The strong interconnection reached inside the core leads to a good FRET.

NT_B700 uptake

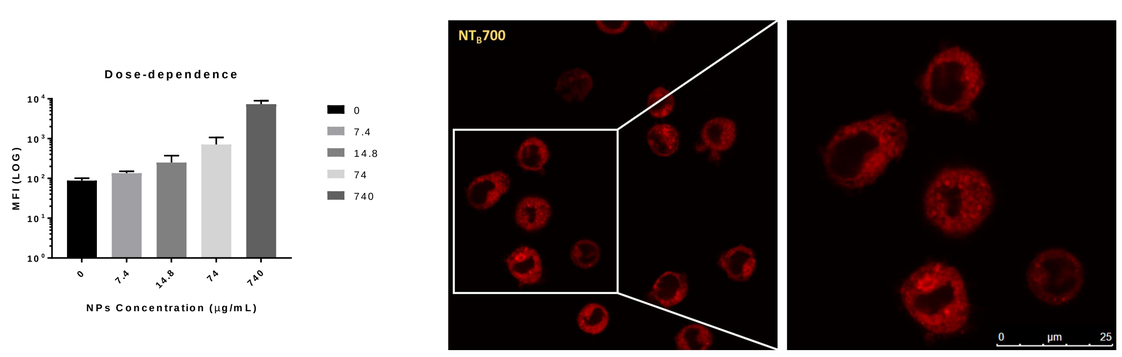
NPs cytotoxicity

U937 cells ($5*10^5$ cells/well) were seeded into 6-well plate and incubated from 1 to 72 hours to evaluate NPs cytotoxicity using trypan blue exclusion assay. There was no significant difference in cell viability between control and cells treated with NT_B700 NPs. Data confirmed by flow cytometry analysis with Propidium Iodide (PI) staining.



NPs uptake measurement

To characterize how NPs are taken up by cells, we quantified their incorporation through flow cytometry, measured by mean fluorescence intensity (MFI). Cytometric experiments were carried out with a FACSCanto™ II flow cytometer (BD Biosciences). NT_B700 NPs were already internalized after 30 minutes of incubation at 37 °C and no differences in terms of MFI under longer incubation times (60', 120', 180') were observed. It has been known that NPs concentration can impact uptake efficiency, therefore we evaluated concentration dependence in NT_B700 NPs uptake. U937 cells were incubated for 1 hour at 37 °C with different concentrations of NPs (7.4, 14.8, 74 and 740 µg/mL). The histogram clearly shows a concentration-dependence.



After 1 hour of NT_B700 NPs exposure, U937 were seeded on MatTek glass bottom chambers (MatTek Corporation) and analyzed by a Leica TCS SP5 II confocal microscope (Leica Microsystem). The overhead images confirmed NPs internalization, with no apparently nuclear involvement. The sharpness observed allows to consider AcZon NPs good candidates as imaging tool, both in flow cytometry and in fluorescence microscopy.

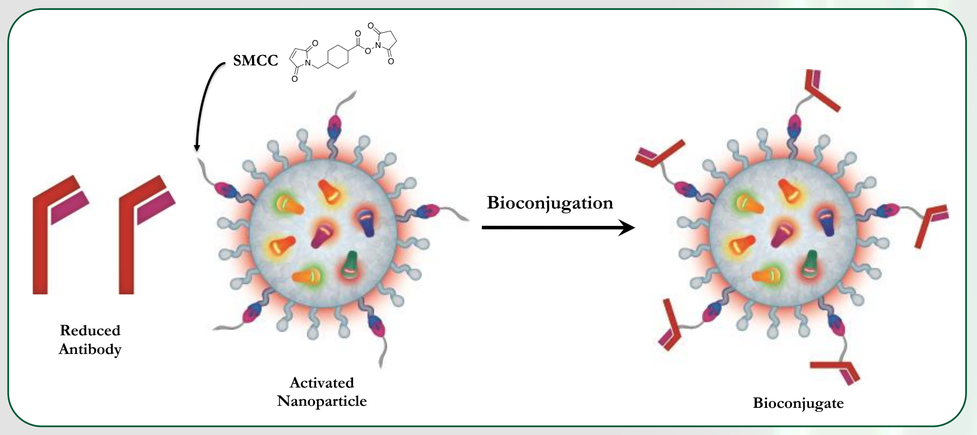
References: C. Pellegrino et al. *J Nanomater Mol Nanotechonology* (2018) S6; Peñaloza et al. *J Nanobiotechnol* (2017) 15:1; SE McNeil et al. *J Leukoc Biol.* (2005) 78(3):585-94; A. Mahapatro et al. *J Nanobiotechnology*. (2011) 28;9:55; JV Jokerst et al. *Nanomedicine* (2011) 6(4):715-28; Z. Cheng et al. *Science.* (2012) 16; 338(6109): 903–910.

NPs bioconjugation

Site-specific conjugation involves a crosslinker reagent (SMCC) containing n-hydroxysuccinimide (NHS) ester and maleimide groups that simultaneously allow covalent conjugation of amine- and sulfhydryl-containing molecules. NHS reacts with the primary amines placed on the NPs surface, while maleimide reacts with sulfhydryl groups available on the hinge region of monoclonal antibodies, avoiding the antigen binding sites.

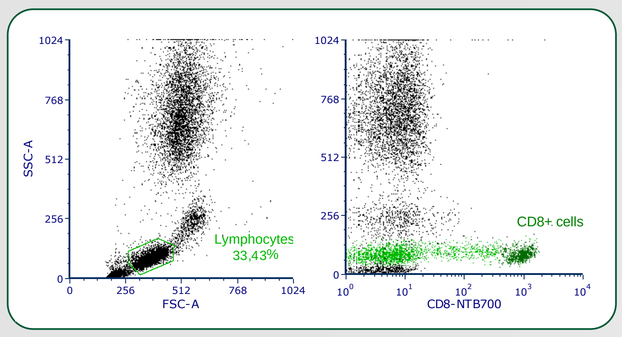
Bioconjugation steps:

- NPs activation with the crosslinker
- Antibody reduction
- Bioconjugation reaction
- Purification by size-exclusion and affinity chromatography



Ab-NPs targeting capability

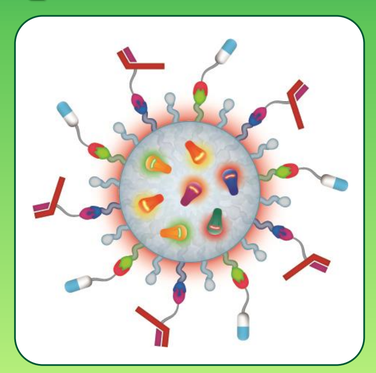
Whole peripheral blood has been stained with Ms. anti-human CD8-NT_B700 (0.0125 mg/mL), erythrocytes lysated with a NH_4Cl solution and samples acquired on the aforementioned flow cytometer.



The alongside dot plots show a significant binding specificity of the Ab-NPs conjugated on the targeted cells. These results and previous data obtained in AcZon prove to assume them a good targeting tool.

Conclusions and future perspectives

Results showed that NT_B700 NPs uptake seems to be a spontaneous, concentration-dependent and rapid process, which doesn't induce significant cytotoxic effects on U937 cell line. Furthermore, Ab-NPs conjugated are able to specifically bind their target on whole peripheral blood. These preliminary data allow to consider AcZon NPs a promising platform for the development of a multifunctional system.



Hypothetical multifunctional AcZon NPs

Exploiting the functional groups on the shell surface, we would conjugate a cytotoxic drug, in addition to the targeting molecule, to combine imaging and therapeutic applications in a unique tool.

Acknowledgements and contact

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