Fluorescent Nanoparticles as tool for next generation ADCs: site specific antibody conjugation strategies for highly pure targeting systems and accurate detection of drug delivery

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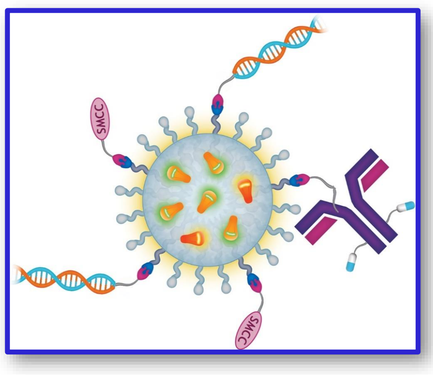
Background

Antibodies are the most rapidly growing drug class and have a major impact on human health, particularly in oncology, autoimmunity and chronic inflammation diseases. Antibody-Drug Conjugates (ADCs) have emerged as targeted therapeutics against cancer by combining the selectivity of the antibody component with the potency of cytotoxic drugs. Although this promising strategy, there are several limitations involving ADCs technology.

To overcome ADCs limitations our proposal is to develop a multifunctional system based on the capability of linking monoclonal antibodies (mAbs) simultaneously to bright fluorescent molecules and drug. Thanks to their unique features of shape, size and charge, AcZon Silica Nanoparticles (NPs) appeared to be good candidates in a wide range of fields. Fluorescent molecules are firstly entrapped into a nanoparticles-based system able to protect them from external stimuli and then conjugated to monoclonal antibodies via crosslinkers, resulting in stable bio-conjugate, which preserves biological properties. This unique approach leads to one antibody per nanoparticle, therefore other ligands sites are still suitable for drug conjugation. Moreover, the design of these systems can be based on a modular approach, making NPs adaptable for different applications. In the present study we describe our bio-conjugation technique, exploiting precise site-specific conjugation and subsequently purifications would yield highly homogeneous, stable and pure mAb-NP conjugate that could improves biological properties of currently used ADCs.

Future Perspectives

Since Aczon NPs can be easily modulated in terms of type and numbers of functional groups exposed on the surface, this promising strategy can be suitable for conjugation different molecules (e.g. mAb, drug, peptides, DNA, radionuclides), taking place in very different applications in medicine.



Schematic representation of multifunctional tool for ADCs improvement with AcZon NPs, involving mAb-drug conjugated to NPs-DNA.

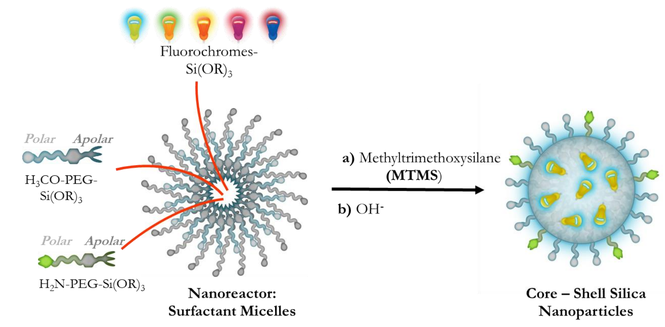
Acknowledgements and Contacts

If you are interested in more information, don't hesitate to visited our web site (www.aczonpharma.com) or contact us: angelavolpe@aczonpharma.com

Bio-conjugation Technique

AcZon Fluorescent Silica Nanoparticles (NPs)

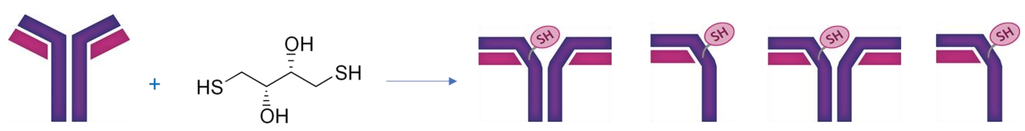
AcZon nanoprobes are core-shell silica nanoparticles synthetized through a micelle-assisted method. The base-catalysed hydrolysis of a silane precursor, along with the dyes and polyethylenglycol *(PEG),* functionalized with a trialkoxysilane group, leads to the formation of fluorescent monodisperse nanoparticles. Thanks to the nanometric dimension, the silica core can hold multiple fluorescent molecules those resulting in increased signal intensity. In the field of fluorescence, silica has proven to be an excellent platform, thanks to the fact that it is photophysically inert and intrinsically non-toxic.



The shell is made of PEG which, besides guarantying stability in water, protects the dyes from external stimuli and ensures a phenomenon known as "stealth effect", useful in drug delivery systems. AcZon NPs can be easily modulated in terms of type and number of functional groups exposed on the surface. Various reactive functional groups necessary to allow subsequent bio-conjugation are easily introduced in the PEG coating (e.g. amine, thiol, carboxyl or methacrylate). NPs used in this work have high concentration of amine reactive groups on the surface which can be linked to monoclonal antibodies via crosslinkers, resulting in site-specific conjugation.

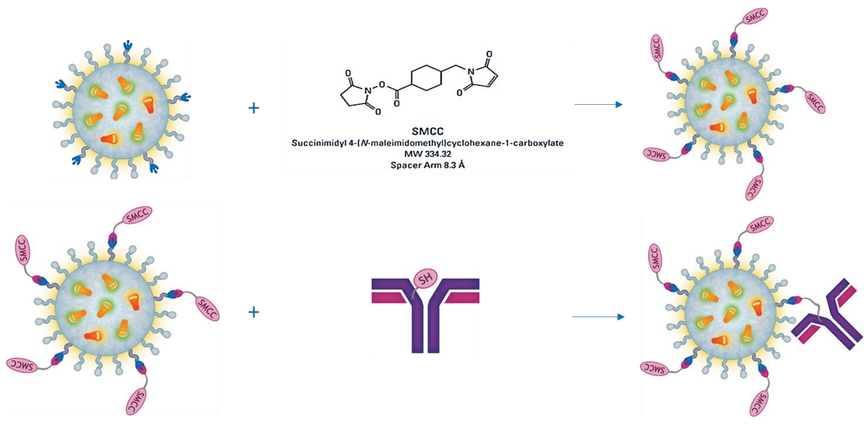
Site-Specific Bioconjugation

The unique structural molecular characteristics of antibodies offer several choices for modification and conjugation schemes. The chemistry used to affect conjugate formation should be chosen to yield the best possible retention of antigen binding activity. Site-specific conjugation involves a crosslinker reagent that can specifically react with residues exclusively on specific positions on the immunoglobulin surface, avoiding the antigen binding sites. The disulphide bridges in the hinge region can be selectively cleaved with a reducing agent such us 1,4-Dithiothreitol *(DTT),* effecting mainly the bonds between the heavy chains of the immunoglobulin. This strategy leads to obtain exposed sulfhydryl groups which can be targeted for conjugation using thiol-reactive probes or crosslinkers, preserving the antibody's biological activity.



Antibodies-Silica Fluorescent Nanoparticles Conjugates

Our bioconjugation method takes advantage of the heterobifunctional crosslinker Succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate *(SMCC)* which contains N-hydroxysuccinimide (NHS) ester and maleimide groups that simultaneously allow covalent conjugation of amines and sulfhydryl containing molecules. NHS reacts with the primary amines placed on the NPs surface, to form amide bonds, while maleimides reacts with sulfhydryl groups available on the hinge region of monoclonal antibodies, to form stable thioester bonds. Molar ratio modulation has been adequate to gain one antibody per NPs, therefore other ligand sites are still suitable for drug conjugation.



Purification Techniques

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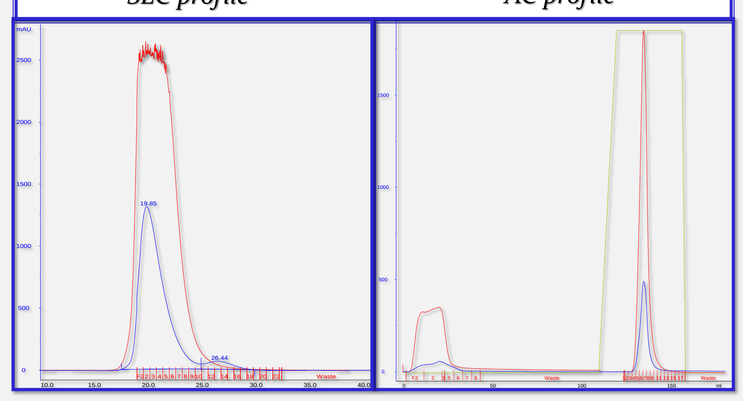
Purification steps involve two chromatography techniques: Size Exclusion Chromatography *(SEC)* separates the unbound antibodies from bioconjugate, while affinity chromatography *(AC)* eliminates unbound nanoparticles, reaching thus a high degree of purity (more than 96%).

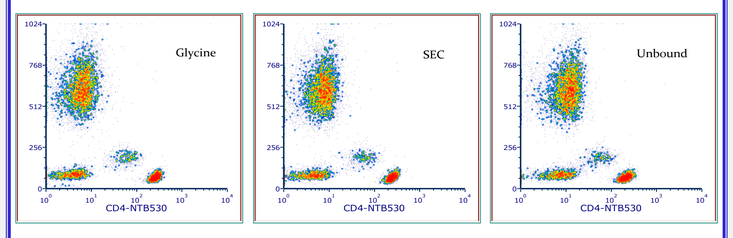
SEC profile *AC profile*



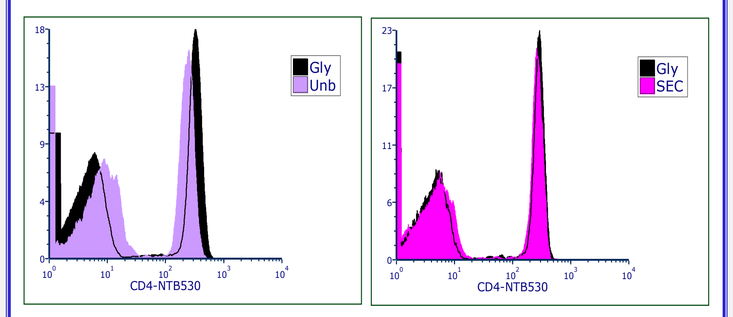
Results

Flow-cytometry evidences

Whole peripheral blood has been stained with Ms. Anti-human CD4-NT_B530 (0,0125 mg/mL), erythrocytes lysated with a NH_4Cl solution and samples acquired on flow cytometer. The dot plots compare anti-CD4 conjugated with NPs NTB530, after purification with SEC and AC.



The dot plots and histograms show that the purification by AC, (glycine), leads to an improvement of the conjugated mAb-NP performance: higher fluorescence (mean positive) and better discrimination (S/B) between the positive and negative population. The sample purified by SEC and the unbound showed instead a lower discrimination (S/B) as we had a higher a-specific contribution (mean negative).



Conclusions

Ab-NP conjugates leads to overcome the classic limits faced with fluorescent molecules, enhancing system homogeneity, purity and making the best performance for both fluorescence intensity and stability. In addition, previous data showed an active NPs internalization into various cell lines and apparently no nuclear involvement, which didn't cause cytotoxic effects.

NPs versatility, associates to conjugations technology, allows considering this system as a promising platform for future development of a multifunctional system, connecting therapeutics to accurate detection in a unique tool.

References: -***Greg T. Hermanson***, Bioconjugate Tecniques, Second Edition;

-***C. Pellegrino et al.***, JNanomater Mol Nanotechnol, (2018) S6; ***Joào P.M. Nunes et al.***, RSC Adv, (2017), 7, 24828-24832; ***Rowan E. Moore et al.***, BioPharm International and LCGC (2018).