Nanoparticle-based immunodetection of the tumor marker CD30

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Introduction
Luminescent nanoparticles are promising tools for a wide range of biological and medical applications. Here we present our recent activities in the fabrication and biofunctionalization of luminescent silica nanoparticles (NP) and in the development of novel nanoparticle-based CD30 immunodetection assays. CD30 is a 120 kDa transmembrane molecule that is overexpressed on activated lymphocytes, hematologic malignancies and inflammatory disorders, such as hepatitis and HIV. The extracellular domain is constitutively sheeded from the membrane. Elevated serum levels of the soluble form sCD30 are regarded as tumor markers for Hodgkin disease and other related disorders.

Synthesis of Luminescent Nanoparticles (NP)

Synthesis of monodisperse, luminescent dye labelled silica NP by sol-gel technology (d = 60 – 160 nm)
Incorporation of various organic dyes into the SiO₂-matrix by covalent attachment:
- increases the resistance to photobleaching
- prevents dye leakage

Surface Modification of Nanoparticles

- Subsequent introduction of reactive functionalities to the surface of NP
- Systematic adjustment of spacer length and type of chemical functionality, depending on the application
- Qualitative and quantitative analysis of NP surface coverage with chemical functionalities

Biofunctionalization and Nanoparticle-based immunodetection of CD30 and sCD30

Application: flow cytometry
Detection: membrane-bound CD30
Flow cytometry binding analysis of CD30⁺ and CD30⁻ cell lines, excitation 488 nm, emission measured by FL-2

Application: confocal microscopy
Detection: membrane-bound CD30
Binding analysis by confocal microscopy CD30⁺ and CD30⁻ cell lines were stained for nuclei (blue) and CD30 (red)

Application: solid phase assay
Detection: soluble CD30
Solid phase assay: serial dilutions of sCD30 containing cell culture supernatant were analyzed by a sandwich ELISA using an antibody with high affinity to the human CD30 gene product antibody NP set-up (blue line)

Conclusion
- Organic dye labelled silica NP have been proved to meet all requirements for different immuno assay applications
- Oriented and non-oriented functional coupling of antibodies and recombinant formats were realized by appropriate chemical surface modifications of the NP

Outlook
- Qualitative surface analysis of coverage density with carboxyl functionalities
- Analysis of cell physiological parameters, e.g. cytotoxicity and internationalization

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