

* Equal contribution

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ABSTRACT

The occurrence of metastases is the main cause of death for breast cancer patients. Micrometastases, which are overall rare disseminated and circulating tumour cells, are present in more than 30% of breast cancer patients without any clinical or even histopathological signs of metastases. Current micrometastases detection techniques are based on immunocytochemical and biochemical methods suffering from low recovery of tumour cells enrichment and observer-dependent interpretation.

The use of the highly fluorescent semiconductor nanocrystals "quantum dots" and nanocrystal-encoded microbeads tagged with a wide panel of the antibodies against specific tumour markers offers unique possibilities for ultra sensitive micrometastases detection with patients' serum and tissues. The nanoparticle-based diagnostics would be able to allow highly parallel quantification of specific proteins in a rapid and low-cost method making a link between the primary tumour and the micrometastases early diagnosis.

PROJECT OBJECTIVES

We propose a breakthrough nano-equipment for rapid, quantitative and parallel detection of micrometastases (MMs) in bone marrow, peripheral blood and lymph node tissue samples from breast cancer patients and to develop applicable *in situ* diagnostics based on unique optical properties of fluorescent semiconductor quantum dots (QDs).

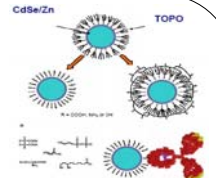
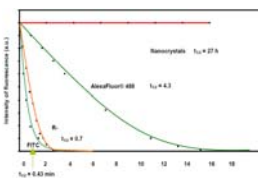
QUANTUM DOTS FOR DETECTION OF MICROMETASTASES

Highly fluorescent QDs are characterized by:

- Large absorption coefficients across a wide spectral range;
- Symmetrical and narrow size-dependent fluorescence emission spectra offering multiplexing advantages;
- Very high levels of brightness and photostability;
- Detection at the single QD level.

Conclusion:

QDs can be used as an emerging tool for analysis of low-abundant biomolecules and rare objects such as MMs.



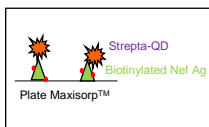
Monodispersed CdSe/ZnS QDs of diameters from 3 nm to 6 nm synthesized and functionalized by our team, are excitable with one wavelength (laser or lamp) but emit fluorescence depending from their size, from blue to red region of spectrum (left). Developed nanocrystals are rock-solid stable against photobleaching (center). As-synthesized QDs consists from the CdSe fluorescing core covered by a 1-2 monolayers of ZnS protecting core fluorescence from quenching; hydrophobic tri-*n*-octyl-phosphin-oxide (TOPO) covers the QDs surface after the synthesis (right). In order to make the nanocrystals biocompatible in aqueous solutions and adaptable for bioconjugation, we have encapsulated QDs within the additional organic shell formed by the mixture of a tri-functional polymers comprising:

- The groups with high affinity to QD surface.
- The hydrophobic chains encapsulating the QD via the strong stacking interaction between these chains.
- The hydrophilic PEG-based functionalities ensuring solubility of nanocrystals and terminating with exact and predefined amount of NH₂- and/or COOH-groups available for bioconjugation.

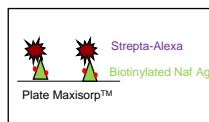
The conjugate of QD with an antibody is shown as an example (right down).

RESULTS

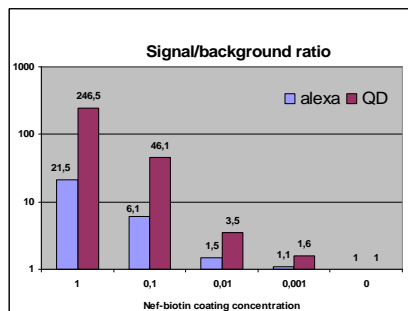
- We have synthesized CdSe/ZnS core/shell QDs of four different fluorescence emission colours (diameters): QD₅₆₅, QD₆₂₀, QD₆₂₅ and QD₈₀₀.
- We have solubilised these QDs with multifunctional polyethyleneglycols and encapsulated them within the organic shell.
- We have compared applications of QDs emitting at 625 nm (QD₆₂₅) and Alexa680® for detection of Nef-protein on solid-state microarrays.
- The antigen (Ag) detection with QD₆₂₅ was found to be 10-fold more sensitive than that with Alexa 680®.
- The detection threshold with Alexa 680® was found to be 1 nM whereas the signal is still detected with QD₆₂₅ in picomolar concentration with TECAN scanner and optimal detection.



Excitation: 230 nm
Emission: 625 nm
QD₇₀₅: 5 nM

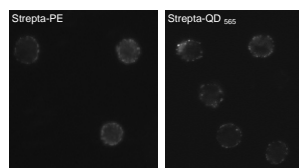
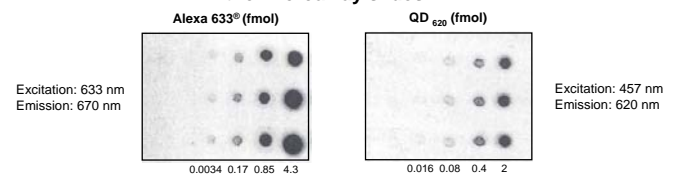


Excitation: 660 nm
Emission: 705 nm
Alexa 680®: 180 nM



A Fluorescence-linked immunosorbent assay (FLISA) test has been done with the scanner TECAN Infinite M200® and the signal/noise ratio for Alexa 680® reaches 20 whereas it rises above 240 for QD₆₂₅.

Comparative fluorescent detection of streptavidin conjugates of Alexa 633® and prepared by us streptavidin-QD₆₂₀: analysis with Typhoon scanner on the microarray slides.



- Antibody anti-CD4 (clone BL4) was purified from the mouse ascit.
- QD₅₆₅ were conjugated with the streptavidin.
- Antibody was biotinylated for further interaction with streptavidin-coupled QD₅₆₅.
- Stainings of PBMC (Peripheral Blood Mononuclear Cells) demonstrated efficient binding of biotinylated antibodies to streptavidinated PE or QDs which was perfectly detectable with fluorescence microscopy.