

Conference Paper

Quantum Dot Conjugates in Functional Imaging and Highly Sensitive Biochemical Assays

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Abstract

Semiconductor quantum dots (QDs) are characterized by orders of magnitude higher multiphoton linear absorption cross-sections compared with conventional organic dyes. Combined with the QD photoluminescence quantum yield approaching 100%, this fact opens new prospects for the two-photon functional imaging of QDs tagged with highly specific recognition molecules, thus permitting high-quality images with a very low autofluorescence contribution to be obtained. Additionally, unique photostability of QDs enables signal accumulation and significant enhancement of the sensitivity of routine biochemical and immunohistochemical assays to be obtained when the conjugates of QDs, instead of organic dyes, are used.

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1. Introduction

The existing photonic techniques of *in vitro* and *in vivo* diagnostics and imaging are mainly limited by the difficulties related to dye photobleaching and their detection in the optically noisy cell and tissue environment [1-3]. Semiconductor quantum dots (QDs) have emerged as alternative tools for cellular labelling, biochemical sensing, probing biocatalysed reactions, and drug delivery [4-7].

2. Materials and Methods

CdSe/ZnS QDs were synthesized as described earlier [6]. Briefly, two solutions were prepared, one containing 10 g of trioctylphosphine oxide (TOPO, Aldrich) and 5 g of hexadecylamine (HDA, Fluka), and the other containing 80 mg of elemental Se and 110

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μl of dimethylcadmium (Strem, 97%) in 1 ml of trioctylphosphine (TOP, Fluka). The first solution was dried, degassed under vacuum at 180°C, purged with argon, and heated to 340°C under argon flow. Then, fast (<1 s) injection of the second solution into the first one yielded CdSe cores approximately 2 nm in size. Further growth of CdSe cores to the desired size (and, hence, the desired fluorescence colour) was induced by prolonged refluxing of the solution at 280°C. After completion of the process, CdSe cores were precipitated at 60°C with methanol, washed twice with methanol, and dried. In order to grow an epitaxial ZnS shell on the CdSe core, a powder of CdSe cores was dissolved in a mixture of 10 g of TOPO and 5 g of HDA. Once again, the mixture was dried and degassed under vacuum at 180°C and purged with argon. A solution containing 210 μl of hexamethyldisilthiane (Fluka) and 130 μl of diethylzinc (Strem, 97%) in 2 ml of TOPO was added dropwise to this mixture at 220°C under argon flow and intense stirring. The resultant colloidal solution of CdSe/ZnS nanoparticles was slowly cooled to 60°C, and the QDs precipitated with methanol were washed twice with methanol and dried. The synthesized QDs consisted of a CdSe core 4.0 nm in diameter and an epitaxial shell of several ZnS monolayers. The QDs were demonstrated to be highly homogeneous in size.

The QDs were solubilised in water as described earlier [6]. Briefly, QDs were first transferred to water after the attachment of DL-cysteine (Sigma) to their surface. The resultant water-soluble QDs displayed a bright orange photoluminescence (PL) with an emission maximum at 570 nm and a quantum yield close to 40% at room temperature. Then, DL-cysteine on the surface of the QDs was replaced with thiol-containing PEG derivatives with carboxyl or hydroxyl group at the end of the polymer chain or with a mixture of 10% of thiol-containing PEG derivatives with amino groups and 90% of thiol-containing PEG derivatives with hydroxyl groups. The samples were incubated overnight at +4°C, pre-cleaned by centrifugation with Amicon Ultra-15 filter units with a 10-kDa cut-off (Millipore), and finally purified from excess of ligands by gel exclusion chromatography on home-made Sephadex-25 (Sigma) columns.

The resultant water-soluble and stable QDs were conjugated with single-domain antibodies as described in Refs. [5, 6] or used in the form of a streptavidine conjugate, as described in Ref. [8]. The absorption and fluorescence spectra of the QDs were recorded using a Tecan Infinite 200 Pro microplate reader; the particle size and zeta potential of QDs were measured by dynamic light scattering using a Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd., Worcestershire, UK).

3. Results and Discussion

As seen in Figure 1, the fluorescent signal from the assay employing strepta-QD obtained in the course of signal accumulation is more than two orders of magnitude stronger than the maximal signals that can be obtained in fluorogenic assays employing the best organic dyes under optimal conditions [8]. This shows that the use of QD-based labels in fluorogenic assays is decisively advantageous.

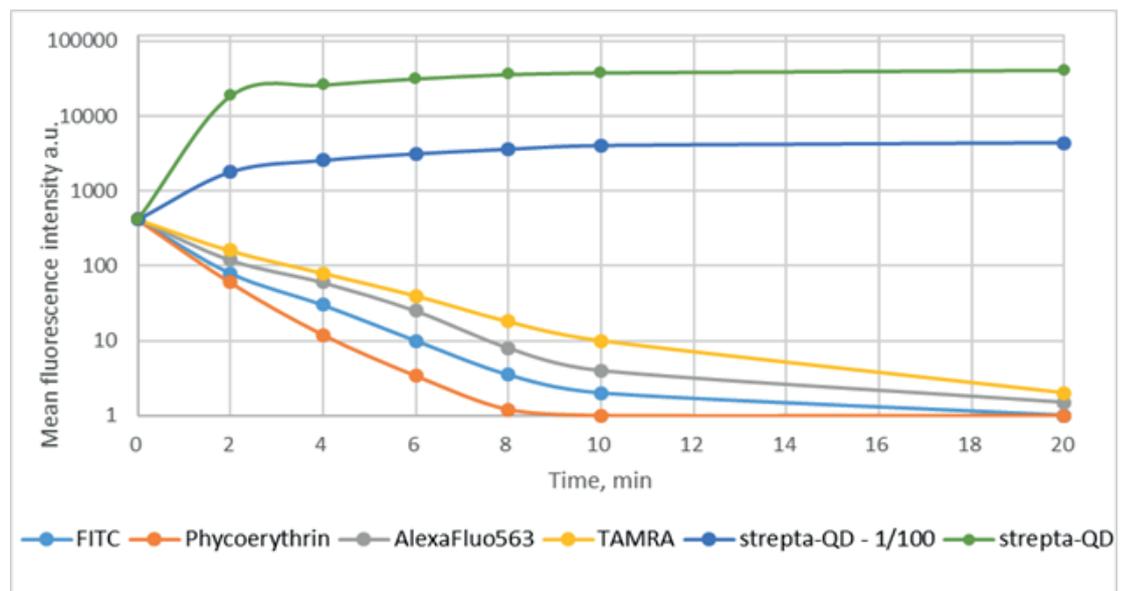


Figure 1: Comparative advantages of semiconductor quantum dots in *in vitro* assays.

Analysis of variations of fluorescent signals as a result of their accumulation for typical *in vitro* biochemical fluorogenic assays employing two concentrations of the fluorescent streptavidin-QD800 conjugate and the most popular organic dyes. Adapted from Ref. [8].

Images of disseminated tumour cells identified by fluorescent microscopy with the single-domain antibody-quantum dot conjugate specific for the HER2 antigen are shown. Adapted from Ref. [10].

Moreover, QD conjugates with single-domain antibodies (sdAbs) have proved to be an efficient tool to detect disseminated human tumour cells and micrometastases by binding the HER2 receptor, an antigen overexpressed in metastatic breast tumour (Figure 2).

Additionally, the better diffusibility of sdAb-QD nanoprobe through tissues facilitates the access to small metastases and complex structures, making them powerful tools for cancer diagnostic and therapeutic applications [9].

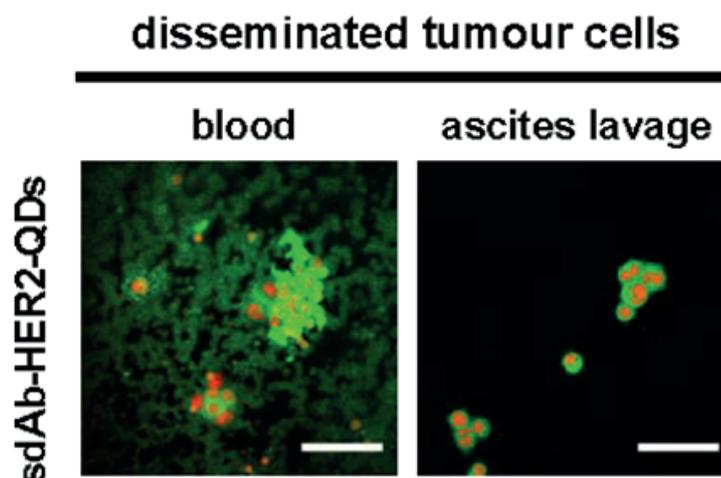


Figure 2: Comparative advantages of semiconductor quantum dots in *in vivo* assays.

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References

- [1] Feofanov, A., Sharonov, S., Fleury, F., Kudelina, I., Nabiev, I., (1997) Quantitative confocal spectral imaging analysis of mitoxantrone within living K562 cells: Intracellular accumulation and distribution of monomers, aggregates, naphthoquinoline metabolite, and drug-target complexes, *Biophys. J.* 73, 3328-3336.
- [2] Feofanov, A., Sharonov, S., Kudelina, I., Fleury, F., Nabiev, I., (1997) Localization and molecular interactions of mitoxantrone within living K562 cells as probed by confocal spectral imaging analysis, *Biophys. J.* 73, 3317-3327.
- [3] Sharonov, S., Chourpa, I., Morjani, H., Nabiev, I., et al., (1994) Confocal imaging analysis in studies of the spatial distribution of antitumor drugs within living cancer cells, *Anal. Chim. Acta* 290, 40-47.
- [4] Bilan, R., Nabiev, I., Sukhanova, A., (2016) Quantum dot-based nanotools for bioimaging, diagnostics, and drug delivery, *ChemBioChem* 17, 2103-2114.
- [5] Montenegro, J.M., Grazu, V., Sukhanova, A., Agarwal, S., de la Fuente, J.M., Nabiev, I., et al. (2013) Controlled antibody/(bio-) conjugation of inorganic nanoparticles for targeted delivery, *Adv. Drug Deliv. Rev.* 65, 677-688.

- [6] Sukhanova, A., Even-Desrumeaux, K., Kisserli, A., Tabary, T., Reveil, B., Millot, J.M., Chames, P., Baty, D., Artemyev, M., Oleinikov, V., Pluot, M., Cohen, J.H., Nabiev, I., (2012) Oriented conjugates of single-domain antibodies and quantum dots: toward a new generation of ultrasmall diagnostic nanoprobe, *Nanomedicine: NBM*, 8, 516–525.
- [7] Rousserie, J., Sukhanova, A., Even-Desrumeaux, K., Fleury, F., Chames, P., Baty, D., Oleinikov, V., Pluot, M., Cohen, J.H.M., Nabiev, I., (2010) Semiconductor quantum dots for multiplexed bio-detection on solid-state microarrays, *Crit. Rev. Oncol. Hematol.*, 74, 1-15.
- [8] Bourguet, E., Brazhnik, K., Sukhanova, A., Moroy, G., Brassart, S., Martin, A., Villena, I., Bellon, G., Sapi, J., Nabiev, I., (2016) Design, synthesis, and use of MMP-2 inhibitor-conjugated quantum dots in functional biochemical assays, *Bioconj. Chem.*, 27, 1067–1081.
- [9] Rakovich, T.Y., et al., (2014) Highly sensitive single domain antibody-quantum dot conjugates for detection of low expression levels of HER2 biomarker in lung and breast cancer cells. *ACS NANO*, 8, 5682–5695.
- [10] Gomes, F.R., Bode, J., Sukhanova, A., Bozrova, S.V., Saccomano, M., Mitkovski, M., Krueger, J.E., Wege, A.K., Stuehmer, W., Samokhvalov, P.S., Baty, D., Chames, P., Nabiev, I., Alves, F., (2017) Single- and two-photon imaging of human micrometastases and disseminated tumour cells with conjugates of nanobodies and quantum dots, *ACS NANO*, submitted.