Fabrication of quantum dot–lectin conjugates as novel fluorescent probes for microscopic and flow cytometric identification of leukemia cells from normal lymphocytes†

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The present study describes a synthesis of QD–lectin conjugates and their application for identification of leukaemia cells from normal lymphocytes using fluorescent confocal microscopy and flow cytometry. The results are compared with commercially available FITC–lectin.

Recent developments in quantum dot (QD) technology have resulted in the introduction of new fluorescent immunocytochemical probes (QDs covalently coupled to immunocompatible biomolecules) for application in ultrasensitive biological detection using fluorescent microscopy and/or flow cytometry.[1–5] In the last 2–3 years the interest in and expectations for this new class of fluorescent markers have expanded enormously, because of their many advantages in comparison with traditional fluorophores. QD-bioconjugates have a higher brightness and about 100 fold higher stability against photobleaching, they are water-soluble and biocompatible, and have the special property of single-source excitation for all colors of QDs, which solves the major problems associated with immunocytochemical analyses.

The present study describes a relatively simple and rapid procedure for conjugation of water-soluble COOH-functionalized CdSe quantum dots with plant-derived lectins and application of QD–lectin conjugates for identification of leukemia cells from normal lymphocytes. The application of QD–lectin conjugates for distinguishing cancer cells from normal ones is based on the ability of some lectins to interact with specific target oligosaccharides on the cancer cell surface and to recognize cancer cells from normal ones (Fig. 1).[6–7] These lectin characteristics make them versatile primary detection reagents in histochemical and flow cytometric detection of cancer cells. Lectin-based technology has been also applied to detect selectively cell surface glycoconjugate ligands and to localize glycoproteins in gels and on protein blots.

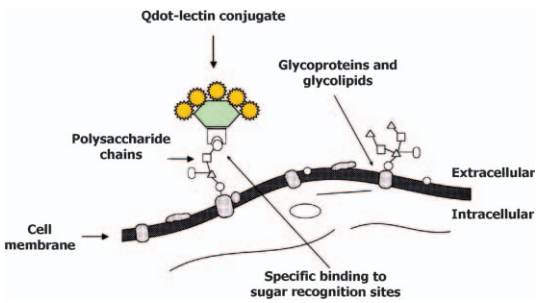

Fig. 1 Scheme of interaction between QD–lectin conjugates and cell surface glycoproteins and glycolipids.

The details of all methods used in this study are described in the ESI.†

The scheme showing QD–lectin conjugation is presented in Fig. 2. Briefly, carbodiimide chemistry was applied using EDC as a zero-length cross-linker. The purification procedure (consisted of several ultrafiltrations using Vivaspin-6/20 filters) aimed to avoid cross-linked lectin molecules. It was established that the conjugation of lectins with quantum dots did not influence their biological properties such as cytotoxicity and cytoagglutinating activity against leukemia cells. Both activities were found to be analogous in the case of QD–lectins or non-conjugated lectins (data are not shown). It is not surprising bearing in mind that the NH_2-groups on the surface of lectin molecule participate in conjugation with the COOH-groups of the water-soluble quantum dots. It is well known that neither amino groups nor sulfhydryl groups participate in the specific binding of lectins to the respective cell surface receptors.[8,9] The degree of lectin-cell binding is considered as a major factor for expression of their biological activity such as cytoagglutination and cytotoxicity.

In our study we conjugated QDs with three different lectins—soybean agglutinin (SBA), *Dolichos biflorus* agglutinin (DBA), and wheat germ agglutinin (WGA). All these lectins were found to possess a high affinity to several leukemia cell lines, without or with low affinity to normal lymphocytes.[9,10] The legume lectins DBA and SBA are tetrameric proteins consisting of similar oligopeptide chains and quaternary structures: DBA—2α2β chains

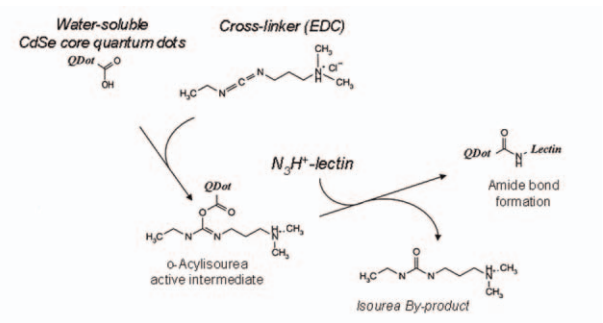

Fig. 2 Conjugation of water-soluble CdSe nanocrystals with lectins—scheme of the experimental protocol.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b4/b419305a/

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(M_r approximately 110 kDa) and SBA—4α chains (M_r approximately 120 kDa). Both lectins have an identical affinity to galactosyl-containing residues of cell-surface receptors. However, it has been established that SBA interacts more strongly with pure carbohydrate ligands than DBA.[11,12] WGA is a dimeric protein and each subunit consists of an assembly of four hevein domains. WGA is considered as *N*-acetyl-D-glucosamine/*N*-acetyl-D-neuraminic acid binding lectin.[11]

QD–lectins were incubated with leukemia cells (Jurkat, derived from acute lymphoblastic leukemia) or normal lymphocytes in RPMI-1640 culture medium at humidified atmosphere (5% CO_2, 37 °C). Aliquots of the cell suspensions were washed three times with PBS to remove free QD–lectin conjugates, and analyzed for QD–lectin–cell interaction using fluorescent confocal microscopy. The results in Fig. 3 show that QD–SBA and QD–DBA bound specifically to leukemia cells (Fig. 3A, green light) and did not interact with normal lymphocytes (Fig. 3B). In the case of normal lymphocytes, no green fluorescence was detected, a direct proof for target-specific interaction of both lectins (SBA and DBA) with leukemia cells only. In contrast, QD–WGA bound non-specifically with both cell types: leukemia cells and normal lymphocytes (Fig. 3A, 3D).

The percentage of leukemia cells interacting specifically with QD–SBA and QD–DBA depended on the cell line. Three more leukemia cell lines were also investigated (MOLT-4, Raji and Daudi) for their interaction with QD–DBA and QD–SBA (data are not shown; however, the microscopic images for Jurkat are representative for these cell lines, too). About 10–35% of leukemia cells (depending on the cell line) did not interact with QD–SBA and QD–DBA (Fig. 3A), probably as a result of different stages of cell differentiation and expression of SBA/DBA-specific galactosyl-containing receptors on the cell surface. Despite this shortcoming, QD–SBA and QD–DBA conjugates can be considered as appropriate fluorescent markers for identification of leukemia cell lines (especially Jurkat, MOLT-4, Raji and Daudi) from normal lymphocytes using fluorescent confocal microscopy. QD–WGA is not an appropriate fluorescent marker for this purpose.

It can be seen also that the fluorescence of leukemia cells interacting with QD-SBA is brighter than the fluorescence of the cells interacting with FITC–SBA (commercially available) (Fig. 3A, 3C). Both substances (QD–SBA and FITC–SBA) were applied to leukemia cells (2×10^6 cells ml^{-1}) in equal concentrations (corresponding to 40 μg lectin ml^{-1}). Moreover, the dynamics of the cell fluorescence during microscopic laser scanning clearly demonstrated that the fluorescence of leukemia cells after their interaction with QD-SBA was stable during 20 min continuous laser scanning (Fig. 4A). Even during 30 min scanning, the quality of the images was relatively good. In contrast, the intensity of cell fluorescence after incubation with FITC–SBA decreased rapidly (Fig. 4B). Five minutes' scanning reduced considerably the quality of the images.

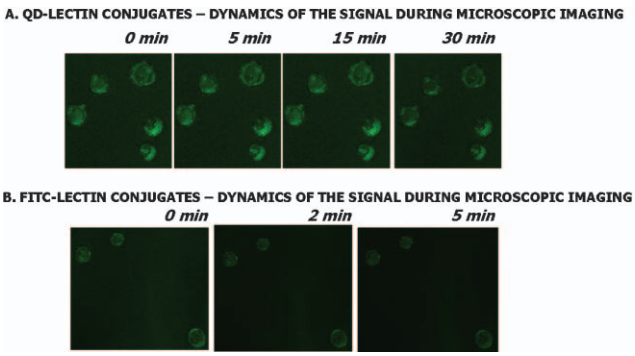

Fig. 4 Fluorescent microscopic imaging of interaction of QD–lectin and FITC–lectin conjugates with leukemia cells: dynamics of the signal during the scanning. The experimental conditions are the same as in Fig. 3.

The long-term incubation of leukemia cells with QD–lectins allowed a detection of their intracellular uptake, as well as an expression of their cytoagglutination activity (Fig. 5). The time-dependent uptake of QD–SBA into leukemia cells Jurkat is shown in Fig. 5A. Unfortunately, from these images it is impossible to clarify the mechanism of QD–SBA delivery into the cells: in some cases endocytosis-like granules were detected on the inner side of the plasmatic membrane.

Twelve hours' incubation of QD–SBA with Jurkat cells resulted in expression of significant cytoagglutination effect. A green fluorescence of many agglutinated cells was detected (Fig. 5B).

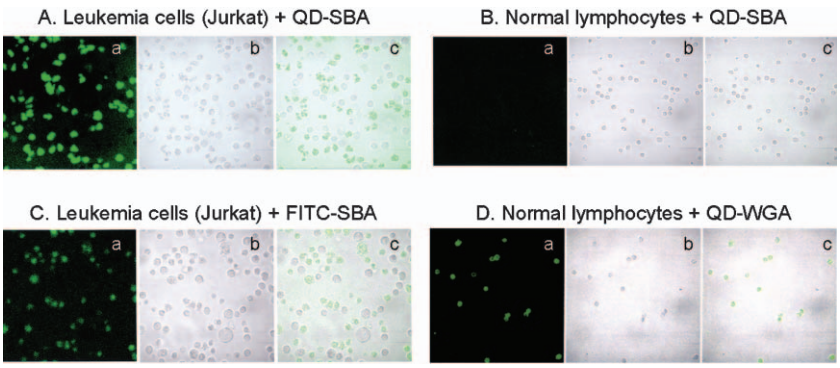

Fig. 3 Fluorescent microscopic imaging of interaction of QD–lectin conjugates with leukemia cells and normal lymphocytes. The images in Fig. 3A are also representative for QD–DBA conjugates. Quadrant (a): fluorescence; quadrant (b): transmission; quadrant (c): fluorescence and transmission. QD–lectins (10 μl, containing 1 mg lectin ml^{-1}) were incubated for 3 h with leukemia cells (200 μl, containing 2×10^6 cells ml^{-1}) or normal lymphocytes (200 μl, containing 2×10^6 cells ml^{-1}) in RPMI-1640 medium, in humidified atmosphere. Aliquots of the cell suspensions were obtained, washed twice by PBS and analyzed by fluorescent confocal microscopy to detect QD–lectin conjugates bound to the cell surface. For comparison, commercially available FITC–SBA (10 μl, containing 1 mg lectin ml^{-1}) were incubated with Jurkat cells (200 μl, 2×10^6 cells ml^{-1}) or normal lymphocytes (200 μl, 2×10^6 cells ml^{-1}) under the same experimental conditions as described above, and the samples were analyzed microscopically. Olympus IX70 microscope was used in all analyses.

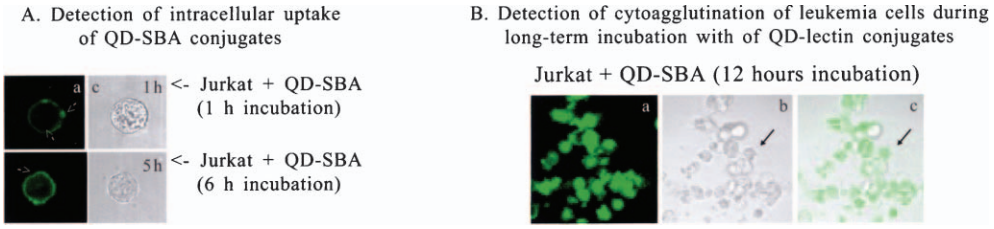

Fig. 5 Fluorescent microscopic imaging of intracellular uptake of QD–lectin conjugates and their cytoagglutination activity in Jurkat cells. QD–SBA (10 μl, containing 1 mg lectin ml^{-1}) was incubated during 1, 6 and 12 hours with leukemia cells (200 μl, containing 2×10^6 cells ml^{-1}) in RPMI-1640 medium, in humidified atmosphere. Aliquots of the cell suspensions were obtained, washed twice by PBS and analyzed by fluorescent confocal microscopy to detect the intracellular uptake of QD–lectin conjugates (A) or their cytoagglutination activity (B). Olympus IX70 microscope was used in all analyses.

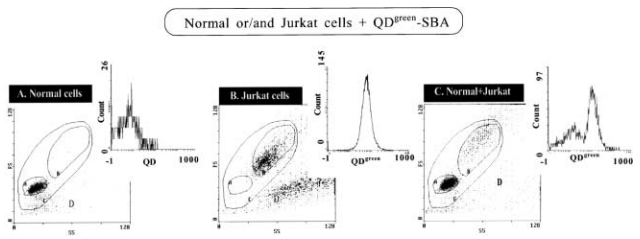

Fig. 6 Flow cytometric analysis of interaction of QD–SBA with leukemia cells (Jurkat) and normal lymphocytes. Quadrant A: viable normal cells; quadrant B: viable leukemia cells; quadrant C: all viable cells; quadrant D: all dead cells. The experimental conditions are the same as in Fig. 3.

The leukemia cells, interacting specifically with QD–SBA, were also distinguished from normal lymphocytes by flow cytometry. The results shown in Fig. 6 demonstrate that in the case of normal lymphocytes self-fluorescence only was detected, while in the case of Jurkat cells a fluorescent peak of QD–SBA appeared in the right site of the histogram. It is possible also to calculate the percentage of leukemia cells in a mixture with normal lymphocytes using their QD–lectin-dependent fluorescence in the histograms (data are not shown).

Summarizing, this study describes a simple procedure for preparation of QD–lectin conjugates in "home-lab" conditions and their application for specific identification of leukemia cells from normal lymphocytes using fluorescent confocal microscopy and flow cytometry.

The QDs used in our study were 3–5 nm in diameter, significantly smaller than the commercially available (usually 20–30 nm, which is commensurate with the size of lectin molecules in quaternary structures). This avoids completely any potential effect of QD on the biological activity of the respective lectin molecule. In the conjugation with lectins, QDs were applied in excess concentration. The described conjugation procedure and the low QD size allow the possibility of obtaining QD–lectin conjugates consisting of one or several QDs per one lectin molecule in contrast to the already published protocols for QD–protein conjugation where several protein molecules are attached to one 20–30 nm size QD-particle. In our conjugates, the QD : lectin ratio was calculated as 1.2 : 1 for SBA and DBA, and 0.8 : 1 for WGA. The QD–lectin conjugates consisting of several lectin molecules per one QD-particle retain a risk of inducing cytoagglutination as a result of cross-links between cells and of compromising the specific cytoagglutination activity of lectins. QD–lectin conjugates, described in our study, avoid this possibility. Moreover, the simple two step purification procedure described in our protocol enables easy removal of non-conjugated QDs and avoids misinterpretations in flow cytometric detection of QD–lectin-labeled cancer cells.

The results clearly demonstrate that QD–SBA and QD–DBA conjugates are appropriate fluorescent markers for identification of several leukemia cell lines (especially Jurkat, MOLT-4, Raji and Daudi cells). QD–lectins give higher quality images and possess higher stability against photobleaching in comparison with commercially available FITC–SBA.

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