Supplementary Material (ESI) for Chemical Communications

Experimental details

Synthesis of CdSe core nanocrystals: CdSe nanocrystals (QDs) were synthesized and purified based on the method of Qu et al.[14] (with modifications). After purification, the vacuum-dried CdSe nanocrystals were dissolved in chloroform and kept in a dark. The nanocrystals were characterized by X-ray analysis, UV- and fluorescent spectroscopy.
Water-solubilization of CdSe core nanocrystals: The water-soluble CdSe core nanocrystals were obtained using mercaptosuccinic acid (Aldrich) as a surface-modifying agent.[15,16] Briefly, 20-30 mg NCs were dissolved in chloroform to obtain OD_{430nm} = 0.5 units and 3 ml dimercaptosuccinic acid (Sigma, dissolved in 150 mM PBS, pH 7.3;) were added to 6 ml of NCs in chloroform. Both phases (water and chloroform) were mixed about 10 min until the water-phase becomes yellow. The mixture was centrifuge for 10 min to separate both phases. The water-phase, containing water-soluble CdSe nanocrystals, was decanted carefully and subjected to ultrafiltration to remove free (non-reacted) dimercaptosuccinic acid. The ultrafiltration was carried out using Vivaspin-6 concentrator (Sartorius) 10,000 MW size. After centrifugation at 3,000xg for 15 min (4 ^{o}C), the lower phase was decanted and subjected additionally to a second ultrafiltration step using Vivaspin-20 concentrator 3,000 MW (3,000xg for 90 min, 4 ^{o}C). The lower phase was discarded and the upper phase was washed three times by 10 ml 150 mM PBS (pH 7.3) on Vivaspin-20 concentrator 3,000 MW following the previous centrifugation conditions. Finally, the upper phase was concentrated and dissolved in PBS to concentration, corresponding to OD_{430nm} = 0.5 (approximately 7.6 μM, calculated by Yu et al.[17]).
The water-soluble nanocrystals showed a bright photoluminescence (quantum yield – 37 %) and a very good stability in a buffer (100-150 mM PBS, pH 7.3, at 4 ^{o}C) – no significant changes in the photoluminescence were detected during three months.
PBS was consisted of 150 mM $Na_2HPO_4x12H_2O$, 150 mM KH_2PO_4, 136 mM NaCl, 2 mM KCl.
Conjugation of water-soluble CdSe core nanocrystals with lectins: The water-soluble nanocrystals were further conjugated with lectin: *Soybean Agglutinin* – SBA (~120 kD), *Dolichos Biflorus Agglutinin* – DBA (~110 kD), or *Wheat Germ Agglutinin* – WGA (~43 kD). Carbodiimide chemistry was applied for conjugation, using N-(3-dimethylaminopropyl)-N`-ethylcarbodiimide hydrochloride (EDC, Sigma) as a zero-length cross-linker (Figure 2). Briefly, 1.2 ml water-soluble nanocrystals (in 150 mM PBS, pH 7.3) were mixed with 150 μl lectin solution (containing 5 mg SBA (or DBA) or 10 mg WGA in 150 μl 150 mM PBS, pH 7.3). Then 150 μl of freshly prepared 6.4 mg/ml EDC stock solution in water was added to the mixture. EDC solution was kept at 4 ^{o}C no more than 30 min before the conjugation procedure. The molar ratio quantum dot:lectin was calculated as follows: 1.8:1 for QD:SBA, 1.7:1 for QD:DBA, and 1.3:1 for QD:WGA).
The samples were incubated 2 hours at RT under shaking in a dark and then kept overnight at 4 ^{o}C. The free non-conjugated nanocrystals as well as the isourea By-product of the conjugation reaction were removed by ultrafiltration using Vivaspin-6. The following ultrafiltration procedure was applied in the case of purification of QD-SBA and QD-DBA: 1.5 ml of the mixture (containing 1.2 ml QDs, 150 μl lectin and 150 μl EDC) were subjected to ultrafiltration using Vivaspin-6 MW 30,000 (3,000xg for 12 min). The lower phase, containing free nanocrystals and isourea By-products, was removed. The

upper phase, containing QD-lectin conjugates, was decanted, diluted to 5 ml by PBS and additionally subjected to ultrafiltration using Vivaspin-20 MW 300,000 (3,000xg for 10 min) to remove cross-linked lectin molecules (if cross-linking happens) holding in the upper phase. The lower phase was decanted and concentrated on Vivaspin-6 MW 30,000 (3,000xg for 12 min) to concentration, corresponding finally to ~1 mg lectin per 1 ml. The protein concentration of QD-lectin conjugates was measured by Lowry.
Analogous procedure was applied for conjugation of QDs with CD90 and CD44 antibodies.
In the case of QD-WGA, the following ultrafiltration procedure was applied: 1.5 ml of the conjugation mixture was subjected to ultrafiltration using Vivaspin-6 MW 10,000 (3,000xg for 15 min). The lower phase, containing free nanocrystals and isourea By-products, was removed. The upper phase, containing QD-WGA conjugates, was decanted and additionally subjected to ultrafiltration using Vivaspin-6 MW 100,000 (3,000xg for 15 min), to remove cross-linked WGA molecules holding in the upper phase. The lower phase was decanted and concentrated on Vivaspin-6 MW 10,000 (3,000xg for 15 min) to concentration, corresponding finally to ~1 mg lectin per 1 ml. The protein concentration of QD-WGA conjugates was measured by Lowry.
Biological activity of QD-lectin conjugates: Two tests were applied to verify the biological activity of QD-lectin conjugates compared to non-conjugated lectins: cytotoxicity test and cytoagglutination test. The cytotoxicity of QD-lectins and non-conjugated lectins against leukemia cells was determined by flow cytometry. The protocol of flow cytometric measurements is described below. The calculation of lectin cytotoxicity was carried out by the following equation:
percentage of dead cells = [1 – quadrant B events/(quadrant C events + quadrant D events)]x100
The details of flow cytometric assay of lectin cytotoxicity are described in Bakalova et al.[16] The cytoagglutination activity of QD-lectins and non-conjugated lectins was determined spectrophotometrically by the method of Ohba et al..[17]
Preparation of cells: The human leukemia cell lines (Jurkat and MOLT-4 - derived from acute lymphoblastic leukemia; Daudi and Raji – derived from Burkitt's lymphoma) were cultured in RPMI-1640 medium supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 100 μg/ml streptomycin, 100 U/ml penicillin in a humidified atmosphere at 37 ^{o}C with 5 % CO_2. The cell lines were a generous gift of Dr. J. Minowada (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan).
The normal lymphocytes were purified from heparinized peripheral blood obtained from clinically healthy blood donors (aged 40-41 years) by Lymphosepar I.
The cells used for assay were in a logarithmic phase. They were sedimented by centrifugation (1000 rpm, 10 min) and washed three times with PBS (Ca^{2+} and Mg^{2+} free, 4 ^{o}C) before experiments.
Fluorescent confocal microscopy: Ten μl of QD-lectin solution (containing 1 mg lectin/ml) were incubated with 200 μl leukemia cells ($2x10^6$ cells/ml) or normal lymphocytes ($2x10^6$ cells/ml) in RPMI-1640 Medium, in humidified atmosphere. At different time intervals 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 or delivered into the cells.

For comparison, 10 μl of commercially available FITC-SBA (1 mg lectin/ml) were incubated with 200 μl Jurkat cells ($2x10^6$ cells/ml) or normal lymphocytes ($2x10^6$ cells/ml) at the same experimental conditions as described above, and the samples were analyzed microscopically.
The type of cells was also improved using adequate fluorescent antibodies – QD^{green}-CD90 for leukemia cells and QD^{red}-CD44 for normal lymphocytes. Ten μl of each antibody were incubated with 200 μl Jurkat cells ($2x10^6$ cells/ml) for 20 min, washed three times by PBS(-) and the cells were analyzed by fluorescent confocal microscopy. Olympus IX70 microscope was used in all analyses.
Flow cytometric assay: The interaction of cells with QD-lectin conjugates or with the respective QD-antibody was also analyzed by flow cytometry, using Beckman Coulter - Epics XL. The flow cytometer was operated in accordance with the manufacturer's recommendations after fine adjustments for optimization. The forward- and side-scatter parameters were adjusted to accommodate the inclusion of both leukemia cells and normal lymphocytes within the acquisition data. No cells were excluded from the analysis, and 10,000 cells were counted. Data were collected and analyzed by using "XL System II" software. Quadrant A in Figures 6 and 7 contains viable normal cells, quadrant B – viable leukemia cells, quadrant C – all viable cells, and quadrant D – all dead cells.

References

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