Journal of Experimental Nanoscience
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t716100757

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Online publication date: 24 March 2010

To cite this Article Liu, Tiantian and Chu, Maoquan(2010) 'Stability of 'quantum dot human epidermal growth factor' bioconjugates prepared using quantum dots synthesised in aqueous solution', Journal of Experimental Nanoscience, 5: 2, 118 — 125
To link to this Article: DOI: 10.1080/17458080903314030
URL: http://dx.doi.org/10.1080/17458080903314030

Journal of Experimental Nanoscience
Vol. 5, No. 2, April 2010, 118–125

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Stability of 'quantum dot human epidermal growth factor' bioconjugates prepared using quantum dots synthesised in aqueous solution

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(*Received 22 April 2009; final version received 6 September 2009*)

As advantaged nanoscale fluorescent bioprobes, quantum dot (QD) bioconjugates have been widely used in biomedical research. However, the stability of these bioconjugates stored over a period of time has been rarely reported. Here, we synthesised water-soluble QDs by conjugating the human epidermal growth factor (EGF) to these low-cost QDs using 1-ethyl-3(3-dimethylamino propyl)-carbodiimide (EDC) as a cross-linking reagent. These bioconjugates nanomaterials were then used to label cancer cells (MDA-MB-435S and SMMC-7721) and normal cells (HL-7702) whose surfaces expressed high-level (for the cancer cells) and low-level (for the normal cells) EGF receptors (EGFR). We found that although the fluorescence intensity of these bioconjugates decreased with prolonged storage time at 4°C for more than 4 months, their aqueous solution retained a transparent appearance, good particle dispersion and very bright fluorescence. Furthermore, the bioconjugates could still well recognise cancer cells. As a control, the normal cells were labelled with a small amount of the fresh and stored bioconjugates. The results presented in this work indicated that QD bioconjugates prepared with QDs synthesised in aqueous solution may be used as a low-cost biomedical test kit for biomedical imaging and diagnosis, where the QD bioconjugates could be stored for long periods of time.

Keywords: quantum dot; bioconjugates; cell labelling; stability

1. Introduction

Quantum dots (QDs) are semiconductor nanocrystals whose radii are smaller than the bulk exciton Bohr radius, constitute a class of materials that is intermediate between the molecular and bulk forms of matter [1,2]. These nanomaterials are currently used in biomedical research areas due to their excellent optical properties compared with the organic dyes, such as broad and continuous absorption, narrow and symmetric fluorescence emission, bright and stable fluorescence [3–5]. Up to now, most of the QDs used in biomedical labelling and imaging have been synthesised in an organic solution because the QDs obtained usually have brighter fluorescence and narrower fluorescence emission then those synthesised in aqueous solution. Unfortunately, toxic and pyrophoric

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ISSN 1745–8080 print/ISSN 1745–8099 online

DOI: 10.1080/17458080903314030
http://www.informaworld.com

organometallic reagents (such as dimethyl cadmium) should be used in the organic synthetic method [6–10]. And before using in biomedical research, QDs must be transferred into the aqueous phase through surface ligand exchange (from hydrophobic to hydrophilic bifunctional ligands). The process is complex, and the quantum yield decreases during the organic-aqueous solutions transfer. Compared with the organometallic approach, aqueous solution synthetic methods [11–18] are safe, simple and low cost. Furthermore, the QDs obtained are usually water soluble, can be directly used for biomedical labelling and imaging, and their fluorescence is bright enough for observation. Using these QDs synthesised in aqueous solution for preparing low-cost biomedical test kits may be of great help to researchers.

During the past few years, uses of the aqueous solution synthetic QDs for *in vitro* and *in vivo* applications have been reported. For specific labelling and target imaging, special biomolecules (such as antibodies or peptides) should be attached to the water-soluble QDs to form fluorescent bioprobes. Some hydrophilic ligands on the QD surface which act as stabilisers should be used for the bioconjugation. Beyond all doubt, if too many of the hydrophilic ligands on the QD surface are reacted with the biomolecules and other organic molecules (such as polyethylene glycol (PEG)), the QD bioconjugates may aggregate and precipitate. However, no work on stabilities towards aggregation, fluorescence or specific bio-labelling after storage over a period of time has been reported for QD bioconjugates prepared using QDs synthesised in aqueous solution. Doing so is important if these low-cost bioconjugates are to be used as a test kit.

In this work, we synthesised red fluorescence-coloured CdTe QDs capped with 3-mercaptopropionic acid (MPA) molecules in aqueous solution, human epidermal growth factor (EGF) was conjugated to the QDs, and used to label two kinds of cancer cells overexpressing EGF receptors (EGFR) and a type of normal cell. We then studied whether the QD bioconjugates retained a transparent appearance, good particle dispersion, bright fluorescence and the ability of well recognising cancer cells after storage at 4°C for several months. 1-Ethyl-3(3-dimethylamino propyl)-carbodiimide (EDC) was used as a cross-linking reagent between QDs and biomolecules, because QD bioconjugates were prepared widely using this reagent. Because many human cancer cells over express EGFR [19], the QD-EGF bio-probe [20] can be used for diagnosis of these cancer cells. The results obtained in this work may be of interest to biomedical researchers and in commercial circles.

2. Experimental section

CdTe QDs were synthesised as follows: fresh NaHTe solution was prepared first by dissolving Te powder into $NaBH_4$ solution (1 : 2 molar ratio) under a nitrogen atmosphere at room temperature. The NaHTe solution was then mixed with a solution of $CdCl_2$ containing MPA (Cd : Te : MPA = 1 : 0.6 : 7 (molar ratio)), and the mixture solution (pH 9.5; adjusted by NaOH) was then heated to 98°C under a nitrogen atmosphere flow for about 2 days. The obtained QD solution was cooled naturally to room temperature and stored in a refrigerator at 4°C.

EDC was purchased from the Shanghai Chemical Reagent Co. Ltd. MPA (purity = 99%) was purchased from the Aldrich Chemical Company. EGF was purchased from the Sigma Co. NH_2-PEG (MW = 5000, purity > 98%) was obtained from the Beijing JenKem Technology Co., Ltd. Phosphate-buffered saline (PBS; pH 7.4) was prepared by ourselves. All reagents were used without further purification.

RPMI-1640 culture medium and foetal calf serum (FCS) were obtained from Gibco (USA). Leibovitz's L-15 medium, human breast cancer cells (MDA-MB-453S), liver cancer cells (SMMC-7721) and normal liver cells (HL-7702) were ordered from the Chinese Academy of Sciences. The water used in all experiments was prepared using an ultrapure water system (Molgeneral220a).

For a typical preparation of the QD bioconjugates, 55 µl of PBS-dissolved EDC (5.94 nmol) was mixed with 30 µl of QDs (0.036 nmol), 30 min later, 50 µl of PBS-dissolved PEG (1.8 nmol) was added, 2 h later, 25 µl of PBS-dissolved EGF (0.18 nmol) was added. The mixed solution was then stored at 4°C overnight (the molar ratio of the QDs to PEG to EGF to EDC was 1 : 50 : 5 : 165). Then, the mixture solution was dialysed against the original QDs solution (without EDC, PEG and EGF) using a 10000 MWCO dialysis tube

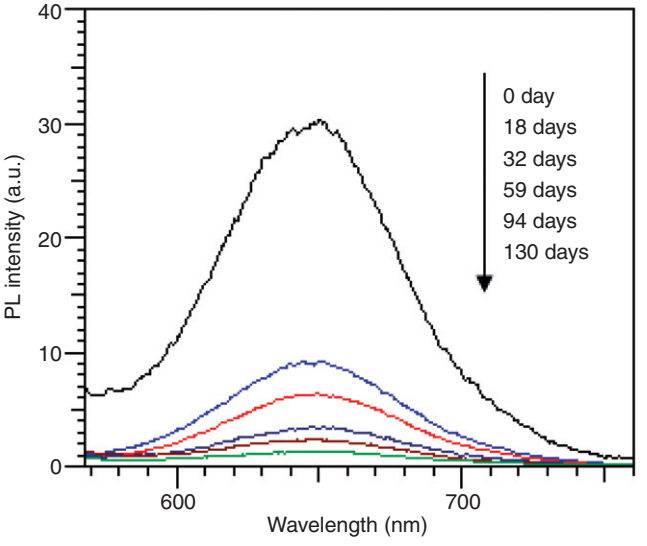

Figure 1. The PL spectra of the QDs bioconjugates varying with time during refrigerated storage at 4°C (excitation wavelength: 400 nm).

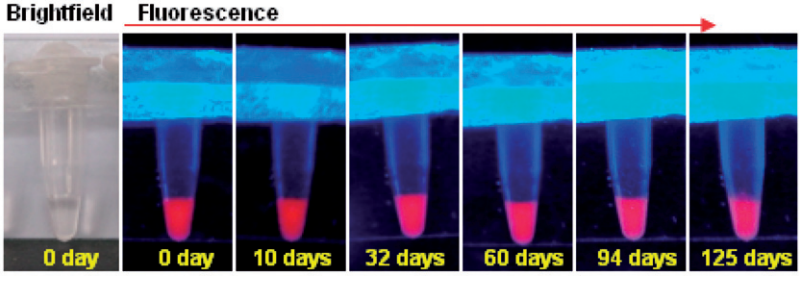

Figure 2. The bright field and true-colour fluorescent images of the QD bioconjugates (containing 225 nmol/L of QDs) after refrigerated storage at 4°C for different lengths of time.

at low temperature (about 16°C) for about 30 min to remove the free molecules. The QD bioconjugate solution obtained was stored in a refrigerator at 4°C.

The room temperature fluorescence spectra of the QD bioconjugate solutions were measured on a fluorescence spectrometer (F-2500 from Hitachi, Japan) with a xenon lamp source. The true-colour fluorescence images were taken using a digital colour camera (COOLPIX4300, Nikon, Japan) that was attached to a UV detector (ZF, Kanghua, Shanghai, China). The morphologies were observed by transmission electron microscopy (TEM; a JSM-6360LV from JEOL, Japan).

MDA-MB-435S cells were cultured in L-15 medium with 10% FCS and 1% antibiotic-antimycotic, at 37°C, in a chamber containing 5% CO_2. SMMC-7721 and HL-7702 cells were cultured in the same conditions as those MDA-MB-435S cells, where only the culture medium was replaced by RPMI-1640. Before incubation with QD bioconjugates, the culture media were removed and the cancer cells (MDA-MB-435S and SMMC-7721) were washed 3 times with PBS. The QD bioconjugates dissolved in the culture media (without FCS; containing 180 nmol/L of QDs) were added to the just washed cells and incubated with the cells at 37°C. One hour later, the cells were washed several times with PBS and imaged with an upright fluorescent microscope (Leica DME, Germany).

As control experiments, QD bioconjugates were incubated with the normal cells (HL-7702), and QDs and the PEG-conjugated QDs (both of them without EGF) were incubated with all the cells, and then washed and imaged at the same conditions as described above.

3. Results and discussion

As shown in Figure 1, the photoluminescence (PL) intensity of the QD bioconjugates (conjugated with PEG and EGF) obviously decrease with prolonged storage time at 4°C, but the maxima emission wavelength is retained at about 650 nm even after storage for more than 4 months. However, we found that the emission intensity of the QDs without conjugating PEG and EGF decreased only slightly, and we also found that the emission

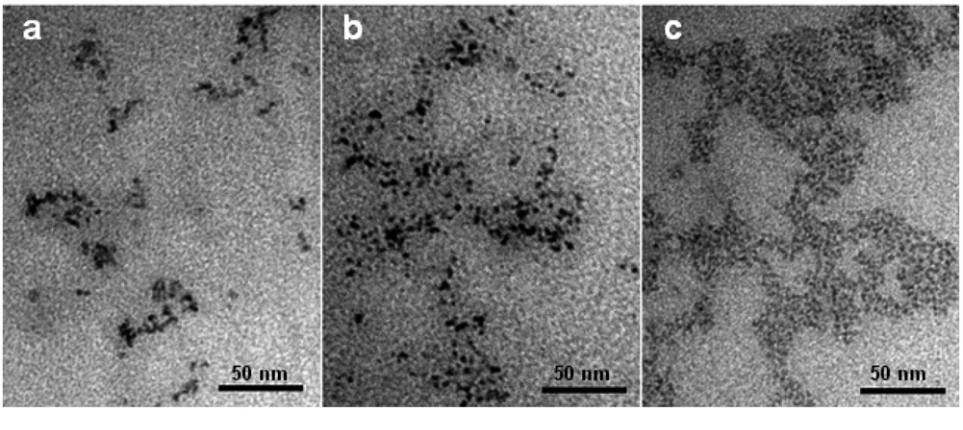

Figure 3. TEM images of the QD bioconjugates after refrigerated storage at 4°C for (a) 3, (b) 63 and (c) 124 days.

wavelength does not shift after storage for several months (data not shown). These results indicate that the fluorescent brightness of QD bioconjugates is not stable compared with that of the original QDs, but the fluorescent colour may be relatively stable.

Although the PL intensity of the QD bioconjugates measured using a fluorescence spectrometer decreased obviously, to our surprise, the true-colour fluorescence of the QDs bioconjugates weakened only slightly in vision with prolonged storage time, and it was still bright red in colour even after storage for more than 4 months (Figure 2). This red fluorescence is bright enough for biomedical labeling and imaging. In addition, the QD bioconjugate solution retains a transparent appearance and no precipitates are found during the storage process.

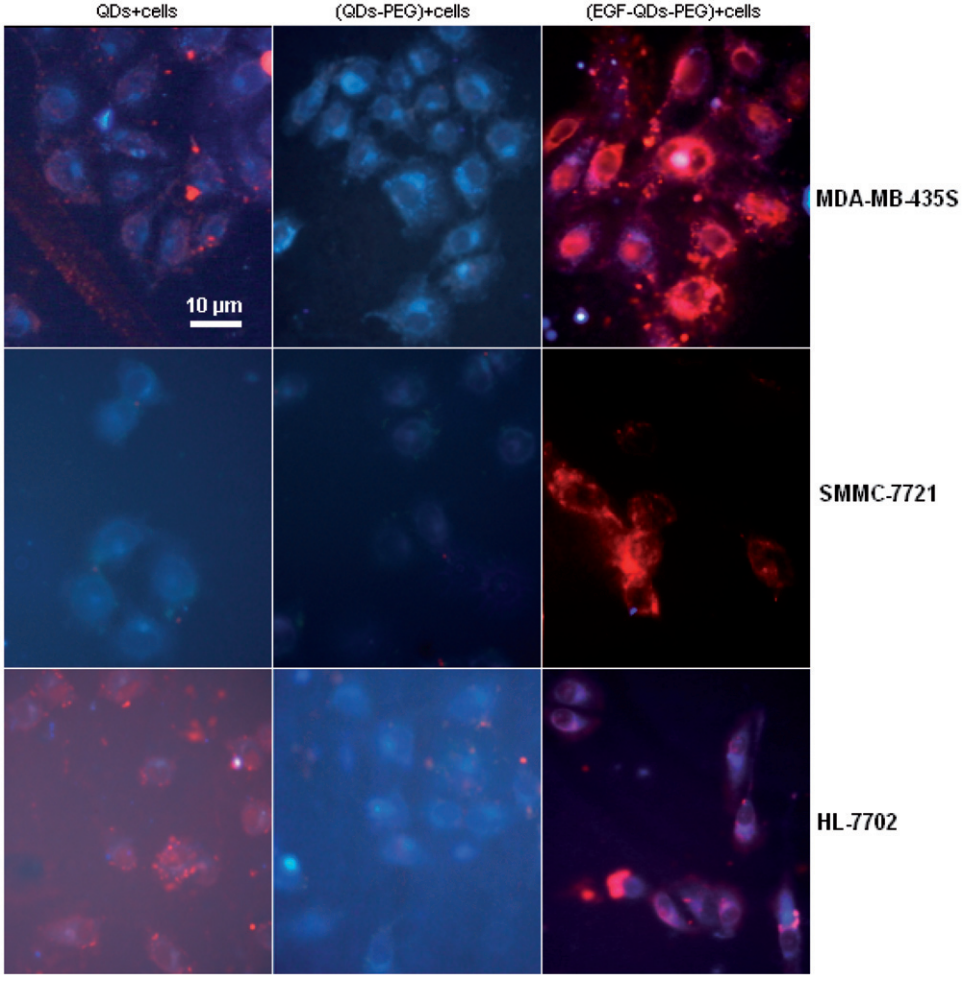

Figure 4. Fluorescent images of MDA-MB-435S, SMMC-7721 and HL-7702 cells labelled with QDs, QD-PEG and fresh QD bioconjugates (EGF-QD-PEG), respectively.

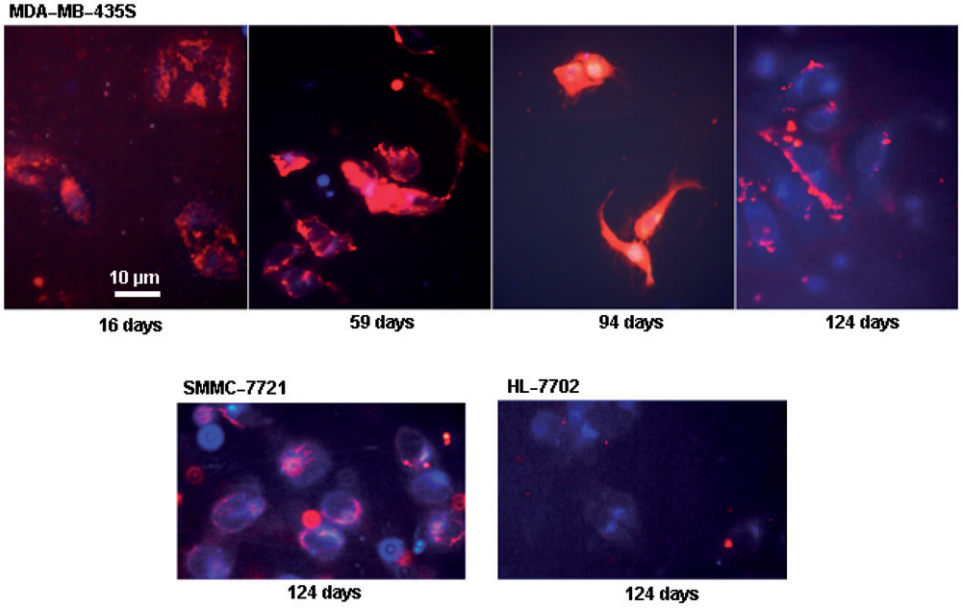

Figure 5. Fluorescent images of MDA-MB-435S, SMMC-7721 and HL-7702 cells labelled with the QD bioconjugates after storage at 4°C for 16, 59, 94 and 124 days, respectively.

The TEM images of the QD bioconjugates are shown in Figure 3. We note that the conjugation of PEG and EGF does not result in aggregation of the QD particles. Even after storage for 124 days, the QD bioconjugates still retain a good dispersiveness. The PEG and EGF cannot be observed clearly by TEM, the dark dots ranging in diameter mainly from 3 to 5 nm are therefore just QDs.

When the QDs without conjugating with PEG/EGF are incubated with MDA-MB-435S, SMMC-7721 and HL-7702 cells, nonspecific binding between the QDs and cells (especially the MDA-MB-435S and HL-7702 cells) can be observed (Figure 4). In general, most of the cells are negatively charged; different cells have different abilities for adsorbing QDs, which may be due to the cells containing different zeta potentials. When the electrostatic repulsion between the QDs and cells is high enough, nonspecific binding may be avoided. In our previous work [21], MDA-MB-435S cells nearly did not adsorb the MPA-capped CdTe QDs, which is different from that in this work, because the zeta potential of the QDs used in this work is lower than those used in the previous work [21]. For most reports, however, polymers should be capped on the QDs to avoid nonspecific binding. In this work, in order to investigate the stability of the common QD bioconjugates, we chose the CdTe QDs with low zeta potential which should be modified with a block polymer–PEG. We have clearly shown that when the PEG-capped QDs are incubated with the above three cells, nonspecific binding is nearly completely removed. We also clearly showed that nearly all the MDA-MB-435S and SMMC-7721 cancer cells are decorated with bright fluorescence after being incubated with the QD bioconjugates for 1 h. Although the normal cells HL-7702 are also labelled with some QD bioconjugates, the

red fluorescence on the cells is obviously less than that on the cancer cells. This is because the EGFR expressed by normal cells is less than that expressed by the cancer cells.

It is interesting that after storage for 16, 59, 94 and 124 days, the QD bioconjugates can still well recognise the MDA-MB-435S and SMMC-7721 cancer cells, and can also occasionally label the HL-7702 cells (Figure 5), which is similar to the ability of fresh QD bioconjugates labelling the three cells. This result indicates that the EGF on the QD surface retains high bioactivity, and the QD bioconjugates are very stable. We are now following up on the stability of these bioconjugates.

4. Conclusions

In summary, PEG and EGF were successfully conjugated onto CdTe QDs synthesised in aqueous solution, and their stabilities after storage at low temperature for several months have been investigated. We found that the QD bioconjugate solution not only retained transparency and bright fluorescence, with the nanoparticles remaining well-dispersed, but also, the QD bioconjugates could well recognise MDA-MB-435S and SMMC-7721 cancer cells even after storage for more than 4 months. The preparation method of this QD bioconjugate sample is simple and low cost. Other antibodies and peptides may be conjugated onto such aqueous solution synthetic QDs by this method for labelling other types of cells. We believe that such QD bioconjugates and their preparation methods may be viable for use in biomedical test kits.

Acknowledgements

This work was supported in part by the 863 National High Technology Research and Development Program of China (No. 2007AA03Z319), the Program for New Century Excellent Talents in University (No. NCET-07-0618), the Nanoscience Foundation of Shanghai (No. 0652nm007) and the National Natural Science Foundation of China (No. 30471965).

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