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PEGYLATED PAMAM ENCAPSULATED InP/ZnS QUANTUM DOT AND THE CELLULAR UPTAKE STUDY

RINGKASAN: *Titik kuantum dengan sifat optik unik telah dikaji secara meluas bagi tujuan perlabelan selular dan 'assay' dalam aplikasi bio-perubatan. Titik kuantum yang mengandungi kadmium telah diketahui sangat toksik terhadap sel hidup akibat daripada pembebasan ion kadmium. Oleh demikian, titik kuantum bebas kadmium adalah diperlukan untuk mengatasi keterbatasan ini. Walau bagaimanapun, keterbatasan keterlarutan titik kuantum hanya dalam pelarut organik seperti n-heksana dan toluena merupakan cabaran bagi penggunaan titik kuantum. Maka, titik kuantum indium fosfat/zink sulfida atau dikenali sebagai titik kuantum InP/ZnS, digunakan bagi menggantikan titik kuantum yang mengandungi kadmium memandangkan ia telah menunjukkan intensiti pendarfluor yang tinggi dan setanding dengan titik kuantum berkadmium. Kaedah pertukaran ligan telah digunakan bagi menjadikan titik kuantum InP/ZnS ini larut di dalam air dan sesuai digunakan untuk aplikasi dalam bidang bio-perubatan. Melalui kerja ini, 'PEGylated Poly(amidoamine)' atau dikenali sebagai 'PEGylated PAMAM', telah disediakan, seterusnya titik kuantum InP/ZnS diperangkap ke dalam rongga PAMAM. Ujian ketoksikan dan pengambilan selular titik kuantum InP/ZnS dan titik kuantum 'PEGylated PAMAM- InP/ZnS' terhadap sel, telah dikaji menggunakan sel kanser dan sel normal. Hasil ujikaji menunjukkan titik kuantum InP/ZnS dan titik kuantum 'PEGylated PAMAM-InP/ZnS' adalah kurang toksik terhadap sel kanser dan sel normal dan menunjukkan pengambilan selular yang baik. Penyesuaian titik kuantum 'PEGylated PAMAM-InP/ZnS' bersama biomarker diharapkan dapat berpotensi untuk digunakan dalam aplikasi pengimejan sel.*

ABSTRACT: Quantum dot with unique optical properties has been extensively studied for the cellular and assay labelling in biomedical applications. The existing cadmium quantum dot was already known as highly toxic towards living cells as the release of cadmium ion will poison the cells. Thus, a free-cadmium quantum dot is needed in order to overcome this limitation. However, it is a challenge to deal with

the solubility of quantum dot as it is only soluble in organic solvent such as n-hexane or toluene. Therefore, indium phosphide/zinc sulfide quantum dot, known as InP/ZnS quantum dot, will be used as it has shown some fluorescent intensity and was comparable to cadmium quantum dot. Due to that, the ligand exchange method was used to prepare water soluble InP/ZnS quantum dot that is suitable for biomedical application. In our work, PEGylated poly(amidoamine), or known as PEGylated PAMAM, was prepared and InP/ZnS quantum dot was entrapped into the internal cavity of the PAMAM. The cell viability and cellular uptake of InP/ZnS qantum dot and PEGylated PAMAM-InP/ZnS quantum dot were studied towards cancerous and non-cancerous cells. The result shows that both samples are less toxic towards these cells. Hence, tailoring this PEGylated PAMAM-InP/ZnS quantum dot with biomarker will be potentially useful for cell imaging studies.

Keywords: Quantum dot, fluorescent, cell viability, cellular uptake, bioimaging

INTRODUCTION

Quantum dot (or known as QD) is a semiconductor nanocrystal composed of heavy metal in the diameter range of 2 nm to 10 nm. The utilization of QD in biomedical imaging has been extensively studied as QD and its properties include highly luminescent, continuous fluorescence lifetime, narrow fluorescence spectra and high resistance to photo-bleaching (Liu *et al.*, 2013). A major drawback which limits the usage of QD in biological applications is the toxicity concern. The most widely used QD consisted of a core of cadmium selenide or telluride, due to their widely quantum confinement region span. However, cadmium ion (Cd^{2+}) is able to bind to the top thiol group of important molecules in mitochondria, which can cause enough damage that leads to cell death (Medintz *et al.*, 2005 and Yong *et al.*, 2012).

Hence, a free-cadmium QD which is less toxic and potentially used for bioimaging is needed. Semiconductor group III-V have become an interest and among them, indium based QD has been highlighted (Yong *et al.*, 2009 and Liu *et al.*, 2013). Soenen *et al.* in his work has found that In-based QD had shown photoluminescence quantum yield of 27 % with low intrinsic toxicity. Ligand exchange of indium phosphide/zinc sulfide (InP/ZnS) QD was done by using mercaptopropionic acid (MPA) to make the QD soluble in water, as it is an important characteristic for a wider biological application. Moreover, molecular conjugate/encapsulate of QD is essential for attachment with desired target molecules such as carbodiimide, maleimide and succinimide for therapeutic and diagnostic application (Wang *et al.*, 2012).

In this work, poly(amidoamine) (or known as PAMAM) dendrimer (generation 3.0) was used to encapsulate InP/ZnS QD. PAMAM with an ellipsoidal or spheroidal shape has a much higher amino group density comparing with conventional macromolecules, possessing empty internal cavities and many functional end groups, which are

responsible for high solubility and reactivity. These specific properties make dendrimer suitable for drug delivery system. Drugs or other molecules can either be attached to PAMAM' end groups or encapsulated in the macromolecule interior. PAMAM also has been chosen in the application of drug delivery device due to its high water solubility, low toxicity, non-immunogenic, and non-antigenic properties (Svenson and Tomalia, 2005). Thus, in order to minimize the side effect due to long term accumulation, surface modification of PAMAM was done by using poly(ethylene glycol) (known as PEG) with molecular weight of 2000.

The preparation and characterization of InP/ZnS QD encapsulated by PEGylated PAMAM (referred as PAMAM-QDs) and their cellular uptake by cancerous and non-cancerous cells are reported. The alamarBlue® cell viability assay was used to evaluate the toxicity of the PAMAM-QDs and the result shows low toxicity on both cancerous and non-cancerous cells.

MATERIALS AND METHOD

InP/ZnS QD (NNCrystal, US Corporation), 3-mercaptopropionic acid (3-MPA; 99 %), PAMAM G3.0 (10 % w/v) (Dendritech, Inc., Midland), methyl-polyethylene 2000 (mPEG, average molecular weight of 200 g mol^{-1}) and HPLC water (Sigma-Aldrich) were used as received. Water soluble InP/ZnS QD was prepared by ligand exchange method with 3-MPA and the final solution was centrifuged at 5000 rpm for 3 min. InP/ZnS QD was finally dissolved in HPLC water and kept at 4 °C for further analysis. Conjugated PEGylated PAMAM was synthesized by reacting the hydroxyl group of PAMAM dendrimer with the mPEG-2000 at a molar ratio of methyl-polyethylene glycol (mPEG) to PAMAM (4:1) as this ratio will give the highest yield percentage of mPEG-PAMAM (Zhu *et al.*, 2009). The conjugated PEGylated PAMAM was dialyzed using membrane dialysis of 14,000 Molecular Weight Cut Off (MWCO) at room temperature for 72 hours. In the presence of N-hydroxysuccinimide (NHS) linker PEGylated PAMAM was mixed with InP/ZnS QD at a ratio of 1:40 and continuously stirred for 72 hours. The mixture was then centrifuged at 10,000 rpm for 5 min using ultrafiltration centrifuge tube 10,000 MWCO and the filtrate was kept at 4 °C.

The molecular structure of the synthesized PEGylated PAMAM was characterized by ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectrum obtained from NMR Spectrometer Bruker Avance III-500MHz using D_2O as a solvent and tetramethylsilane (TMS) as an internal reference. Whilst, the High Resolution Transmission Electron Microscopy (HRTEM) images of water soluble InP/ZnS QD and PAMAM-QDs were obtained from the TECNAI 20 using an acceleration voltage of 200 KV (FEI, USA).

Cell viability study of InP/ZnS QD and PAMAM-QDs against non-cancerous cell, mouse fibroblast cell line (L929) and cancerous cell, histiocytic lymphoma cell line (CRL 1593) were evaluated using alamarBlue® cell viability assay. Cells were seeded at 3 x 10^4 cells/well and maintained in Dulbecco's modified Eagle's medium (DMEM,

Sigma Aldrich) supplemented with 10 % fetal bovine serum (FBS). The cells were cultured at 37 °C in a humidified atmosphere containing 5 % CO_2. After 24 hours, five concentrations of samples were added in thrice ranging from 0-25 µg/ml and continually incubated. After 24 hours, 10 µl of the alamar blue solution was added to each well and left for 4 hours to react. The optical density of the sample was measured using a universal microplate reader (EL800, Bio-Tek Instruments. Inc.). The viability of the cells was determined by normalizing the absorbance of the samples with that of the control's well, assigning the viability of control is 100 %.

For in vitro cell fluorescence imaging, a Confocal Laser Scanning Fluorescence microscope (SPX800, Carl Zeiss, Germany) with laser excitation at 488 nm was used. L929 and CRL1593 cells were seeded at 3 x 10^5 cells/well and maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10 % fetal bovine serum (FBS). The cells were cultured in a humidified atmosphere containing 5 % CO_2. After 24 hours, InP/ZnS QD and PAMAM-QDs were added and incubated. The cells were washed with PBS and fixed with ice-cold methanol for 15 minutes before imaged.

RESULTS AND DISCUSSION

The conjugated PEGylated PAMAM after dialyzed was confirmed by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of PEGylated PAMAM was as shown in Figure 1. Based on the spectrum, it can be seen that the chemical shifts are: δ 3.2 ppm and 3.5 ppm (PEG, -CH2-); 2.3 ppm, 2.5 ppm, 2.7 ppm and 3.3 ppm (PAMAM, -CH2-). The multiple peaks arising between 2.30 ppm to 3.4 ppm are assigned to be the methylene protons of branching units in the dendrimer. The single peak at 3.2 ppm is referring to the methyl protons at the end of PEG. Whilst, the peak at 3.5 ppm is assigned to the methylene protons in the repeating units of PEG (Yang *et al.*, 2008 and Zhu *et al.*, 2009).

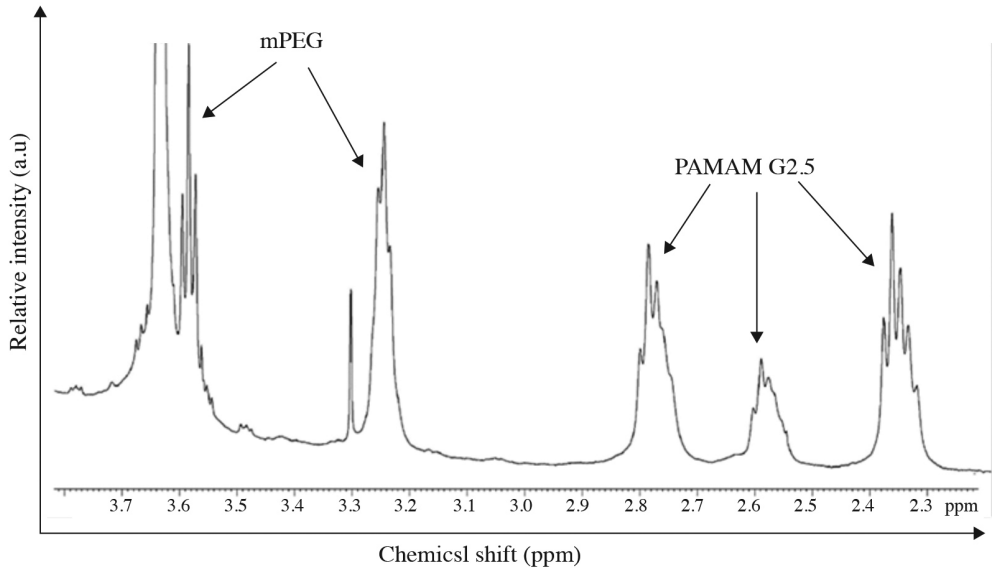

Figure 1. *^{1}H NMR spectrum of PEGylated PAMAM*

HRTEM was then used to visualize InP/ZnS QD after being reacted with PEGylated PAMAM. The HRTEM images of InP/ZnS QD and PAMAM-QD are shown in Figures 2(a) and (b), respectively. From the images, InP/ZnS QD was assumed to be localized in the internal cavities of the PEGylated PAMAM. The average particles size of the assumed QD was measured to be around 5 nm as shown in Figure 2(a). As for Figure 2(b), the assumed InP/ZnS QD was found to be encapsulated/entrapped in the cavities of the PEGylated PAMAM. This result probably due to the intrinsic flexibility of PAMAM structure. Whilst, the smaller image on the right side shows the full image of PAMAM-assumed QD with the diameter size of 100 nm. There might be an agglomeration of PEGylated PAMAM due to the high ratio surface volume as the average size of PAMAM was 5 nm. The assumed encapsulated QD was postulated based on the strong fluorescence images (Figure 4).

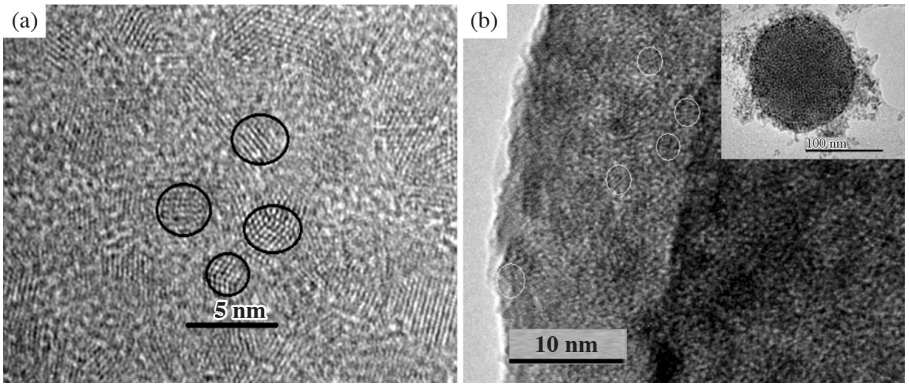

Figure 2. *HRTEM images of (a) InP/ZnS QD at scale bar of 5 nm and (b) PAMAM-QD at scale bar of 10 nm and the smaller image on the right side shows the HRTEM overview of PAMAM-QD at scale bar of 100 nm.*

Figure 3 shows the result of the alamarBlue® cell viability study of L929 and CRL1503 cells treated with InP/ZnS QD and PAMAM-QD. The viability of the cells was found to be maintained above 80 % at the concentration of 0-25 µg/ml after 24 hours of incubation. This study also revealed that PAMAM-QD has a slightly greater cell viability compared to InP/ZnS QD only. This result highlighted the role of PEGylated PAMAM in minimizing the toxicity of InP/ZnS QD.

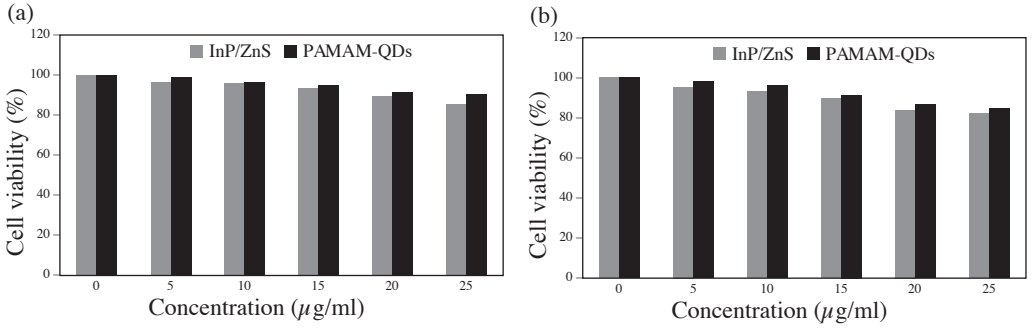

Figure 3. *The viability of (a) L929 and (b) CRL1593 cells after being treated with InP/ZnS QD and PAMAM-QD*

For the cellular uptake study of InP/ZnS QD and PAMAM-QD by L929 and CRL1593 cells, the fluorescent images are shown in Figure 4. Based on the images, it can be seen that InP/ZnS QD has shown strong fluorescence signals and the intensity of fluorescence signal decreased for PAMAM-QD. The decreasing of the fluorescence signal for PAMAM-QD might be due to the entrapment of the InP/ZnS QD in the cavity of the PAMAM. Hence, InP/ZnS QD encapsulated by PEGylated PAMAM do not lose their luminescent properties indicating that PEGylated PAMAM does not interfere with the fluorescence emission of InP/ZnS QD. However, in comparison to InP/ZnS QD which spread over the cells, PAMAM-QD was found to be localized in the nucleus of the cells.

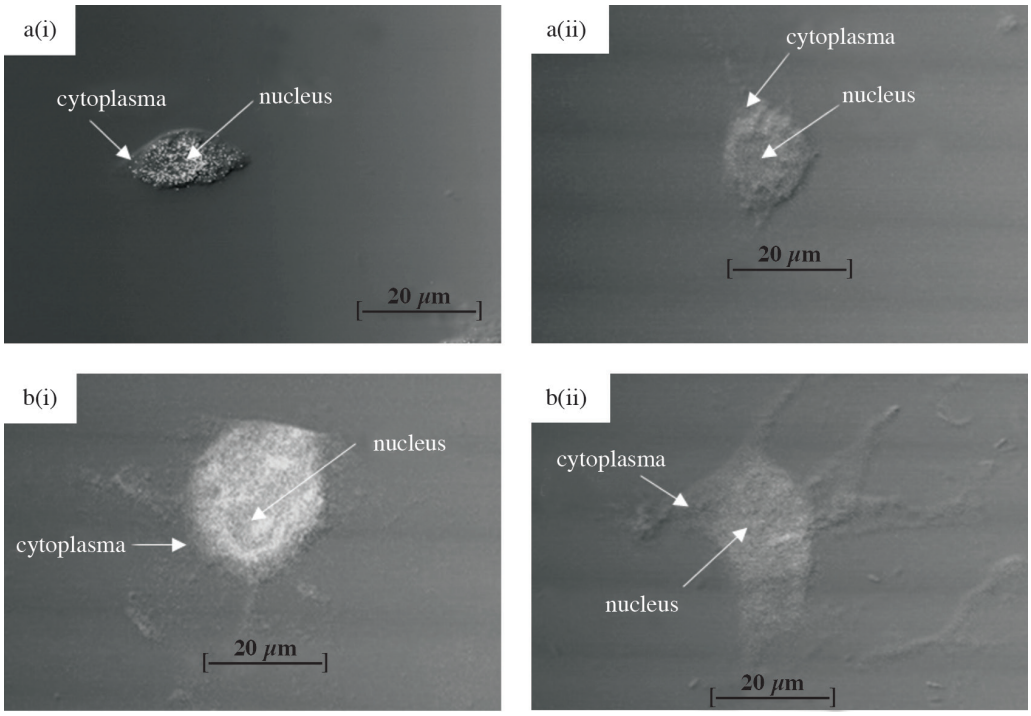

Figure 4. *Confocal fluorescence images of (a) L929 cells treated with (i) InP/ZnS QD and (ii) PAMAM-QD, and (b) CRL1593 cells treated with (i) InP/ZnS QD and (ii) PAMAM-QD*

CONCLUSION

The cell viability study has shown that the prepared PAMAM-QD has a low toxic effect towards cancerous and non-cancerous cells, and the cellular uptake of the cells can be visualized clearly. Therefore, this PAMAM-QD could be useful for biological imaging.

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