Intracellular dynamics of oligopeptide-modified phospholipid polymer nanoparticles

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Statement of Purpose: To obtain a stable and highly sensitive bioimaging fluorescence probe, polymer nanoparticles embedded quantum dots (QDs) were prepared and the surface of them was covered with biocompatible phospholipid polymer. Semiconductor nanocrystal, QDs are promising alternative to conventional organic dyes for bioimaging, because the QDs have novel properties such as optical tunability, improved photostability, narrow photoluminescence spectra, and simultaneous multi-color emission. However, their use has been strongly limited by difficulties in obtaining biocompatibility of the QDs. The polymeric nanoparticles embedding QDs covered with phospholipid polymer showed complete resistance to cellular uptake from HeLa cells owing to the nature of the phosphorylcholine groups. On the other hand, when an arginine octapeptide (R8), which was one of the cell penetrating peptides, was immobilized at the surface of the nanoparticles, they could penetrate the membrane of HeLa cells effectively. Cytotoxicty of the polymeric nanoparticles was not observed even after immobilization of the R8. Fluorescence intensity of the nanoparticles was not bleached during 30 h-continuous observations with fluorescence microscopy. Thus, we obtained stable fluorescent polymer nanoparticles for excellent bioimaging probe.

Methods: Poly(2-methacryloyloxyethyl phosphoryl-choline (MPC)-*co*-*n*-butyl methacrylate (BMA)-*co*-*p*-nitrophenyloxycarbonyl tetraoxyethylene methacrylate)s (PMBN) and poly(lactic acid) (PLA) were used for preparation of nanoparticles containing ZnS-overcoated CdSe QDs (PMBN/PLA/QD). The R8 was immobilized on the nanoparticles (R8-PMBN/PLA/QD) for evaluating the long-term photostability in Hela cells using a confocal laser microscope (CFMS).

Results: The PMBN/PLA/QD showed good dispersibility in aqueous medium including cell culture medium. The morphology of the PMBN/PLA/QD was sphere and its diameter was 10 nm determined with AFM. The surface chracterization with XPS demonstrated that the surface was completely covered with the MPC units. The optical properties of QDs did not alter even after incorporation of them into the polymer nanoparticles. The R8-PMBN/PLA/QD for evaluation of dynamics of nanoparticles in cells. It has the ability to translocate through cell membranes in a manner that does not involve the typical endocytic pathways of internalization. The R8-PMBN/PLA/QD clearly was associated with the HeLa cells and was found internalized at a perinuclear location after 2 h incubation. During 30 h-contunuous observation with the CFMS, the R8-PMBN/PLA/QD internalized in cell

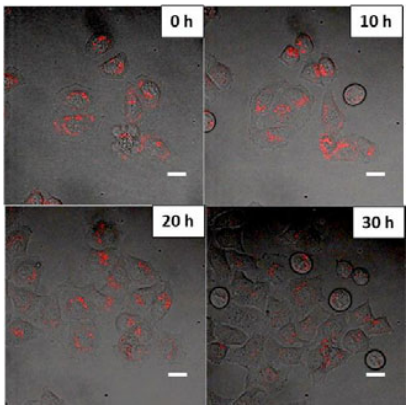

Fig. 1. The time-dependent CFMS images of HeLa cells treated with R8-PMBN/PLA/QD nanoparticles after incubation for 2 h and rinsed. The scale bars are 20 μm.

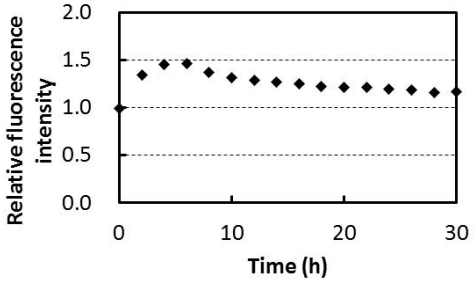

Fig. 2. The fluorescence intensity ratio of R8-PMBN/PLA/QD to their image backgrounds vs time.

were separated into two daughter cells by cell division and the amount of R8-PMBN/PLA/QD in a cell decreased (Fig. 1). Also, the total fluorescence intensity derived from R8-PMBN/PLA/QD in the cells was unchanged for 30 h (Fig. 2). The nanoparticles did not release even when the cells made prolifeation. Also, the fluorescence property could be mainteined for long term. Thus, the R8-PMBN/PLA/QD has strong potential for bioimaging in the dynamics in the cells.

Conclusions: We prepared well water-dispersed and biocompatible QDs using a phospholipid polymer. PMBN/PLA/QD retained the photoproperty of QDs. The PMBN/PLA/QD is good probe for long-term observation of biomolecular dynamics in cells.

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References: K. Ishihara, et al. *Biomacromolecues* 2008, **9**, 3252; K. Ishihara, et al. *Nano Today* 2010, **6**, 61.