Novel stable fluorescence nanoparticles covered with biocompatible phospholipid polymers

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Statement of Purpose: To obtain a stable and highly sensitive bioimaging fluorescence probe, polymer nanoparticles with embedded quantum dots (QDs) were prepared and the surface was covered with biocompatible phospholipid polymers. Also, the oligopeptide was immobilized on the phospholipid polar group platform as a bioaffinity moiety, whose structure seemed artificial cell membrane. Semiconductor QDs are promising alternative to organic dyes for biological imaging. QDs have novel properties such as optical tunability, improved photostability, narrow photoluminescence spectra, and simultaneous multi color emission. But their use has been limited by difficulties in obtaining QDs that are biocompatible. The nanoparticles covered with phospholipid polymers showed resistance to cellular uptake from HeLa cells owing to the nature of the phosphorylcholine groups. When arginine octapeptide (R8), which was one of the cell penetrating peptide, was immobilized at the surface of the nanoparticles, they could penetrate the membrane of HeLa cells effectively. Cytotoxicty of the nanoparticles was not observed even after immobilization of oligopeptide. Thus, we obtained stable fluorescent polymer nanoparticles for excellent bioimaging probe and as a novel evaluation tool for oligopeptide functions in the target cells.

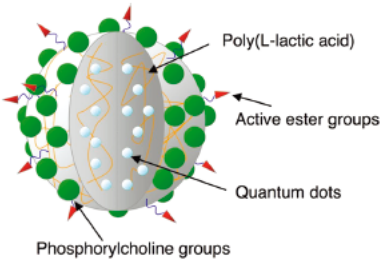

Fig. 1. Fluorescence nanoparticles covered with phospholipid polymer.

Materials and Methods: 2-Methacryloyloxyethyl phosphoryl choline (MPC), *n*-butyl methacrylate (BMA), *p*-nitrophenyloxycarbonyl tetraoxyethylene methacrylate (MEONP) were used to synthesize poly(MPC-*co*-BMA-*co*-MEONP) (PMBN) by a conventional radical polymerization technique. Phospholipid polymer nanoparticles containing ZnS-overcoated CdSe QDs were prepared by solvent evaporation technique. The QDs were suspended in dichloromethane with poly(L-lactic acid) (PLA). The QDs solution was dropped into aqueous PMBN solution. The mixture was sonicated and kept under reduced pressure to evaporate the dichloromethane. The nanoparticles were precipitated by ultracentrifugation. The PMBN/PLA-QDs were resuspended in phosphate buffered saline (PBS). Characterization of PMBN/PLA-QDs was performed using fluorescence spectroscopy, X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM). Various octapeptides and glycine (control) were immobilized on the nanoparticles for evaluating the functionality of the oligopeptides. The nanoparticles were applied to HeLa cells.

Results: The optical properties of QDs were unchanged after incorporation of them into the polymer nanoparticles. The R8 was immobilized to the PMBN/PLA/QD (R8-PMBN/PLA/QD) for evaluation of its cell penetrating ability. It has the ability to translocate through cell membranes in a manner that does not involve the typical endocytic pathways of internalization. A fluorescence microscopy image of HeLa cells that had been incubated with R8-PMBN/PLA/QD (green-emitting) is depicted in Fig. 2b bottom. The R8-PMBN/PLA/QD clearly was associated with the HeLa cells and was found internalized at a perinuclear location. We observed a small amount of cellular uptake only in the cases of octapeptide of glutamic acid(E8) and asparagine(N8). Other octapeptides of tyrosine(Y8) and histidine(H8) were not effective in causing uptake to the cells. The results indicated that R8 could function as a cell penetrating peptide even in close proximity to phosphorylcholine groups, which reduce a translocation into cytosol. Thus, R8-PMBN/PLA/QD is a most suitable material for conducting the kinetic analysis of cell membrane permeation.

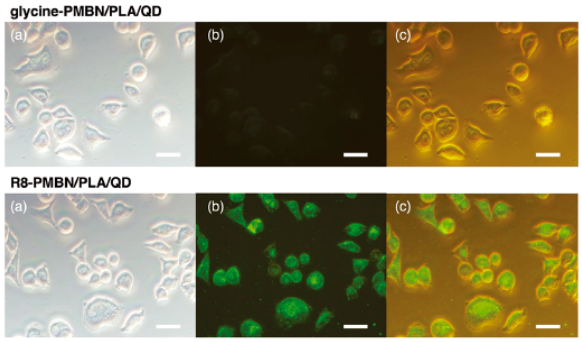

Fig. 2. Microscopic observation of HeLa cells after contact with nanoparticles for 24h. (a) phase contrast image, (b) fluorescence image, (c) merged image. Bar indicates 10 μm.

Conclusions: We have fabricated polymer nanoparticles with embedded QDs in the core, covered with an artificial cell membrane. Although the nanoparticles were fundamentally inert against nonselective cellular uptake from cells, when bioactive molecules were immobilized, the nanoparticles showed excellent affinity to the cells. We conclude that the nanoparticles are candidates for the role of stable and highly sensitive fluorescence bioimaging probes in the field of biotechnology.

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