博士論文（要約）

Functional Well-defined Phospholipid Polymer Biomaterials for Surface Modification of Fluorescence Nanoparticles

（蛍光ナノ粒子表面を修飾する
機能性リン脂質ポリマーバイオマテリアル）

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To elucidate the molecular reactions in cell engineering, establishment of a method of modulation is required. Most notably, the changes of intracellular pH play the important roles in cellular processes and organelle regulation, such as cell cycle progression and the structural stability and function of proteins. All that action going on is made possible by the enzymes. Enzymes will do their job in the range of optimal pH in the cell environment, at which enzymes activities are greatest. Therefore, the tracking method for intracellular pH is vital to the understanding of intricate cellular functions.

This study focuses on the substance kinetics during endocytosis process. The objective of this study is the development of multifunctional nanoparticles for tracking real time intracellular pH modulation via surface modification of quantum dots (QDs) with well-defined polymer.

The well-defined polymers composed of designed monomer compositions, molecular architectures and interested functions have attracted attentions for development of the strategies for much study in the biomaterials, such as surface modification for biosensors. The surface modification of specific structure is necessary for the further applications in the biology.

The QDs with the size range from 2 nm to 10 nm are famous fluorescence probes, and distinguish themselves in unique and outstanding optical properties. QDs are semiconductor nanocrystals. Their fluorescence properties are dependent on the size of the nanocrystals and their adsorption spectra are broad. Thus, the application of QDs is mainly active in bioimaging. However, the market available QDs

as the bioimaging probes still have some problems. Their exploitations are limited because of not only the cytotoxicity, but also the low dispersion ability and instability in aqueous media. On the other hand, in the study of cells, the low molecular weight fluorescence dyes performed very fast cellular uptake, and they entered cytosol directly. The information about organelles was lost. To overcome these problems for exploit the further possible applications in the biology, surface modification of the QDs is necessary.

The living radical polymerization, that is, reversible addition-fragmentation chain transfer polymerization (RAFT) represents key molecular design strategies for polymers by altering the monomer unit sequence, composition and molecular weight. This study improves the design and generates a novel polymers endowed a fine structure, well dispersion ability in aqueous medium, and cytocompatibility as a surface modifier for the QDs. What's more, the controllable molecular architecture, stimuli responsiveness and easy conjugation of target biomolecules of the functional polymers would not only protect life safety of cells but also be helpful to exploring the applications of QDs in the intracellular organelles fields.

In order to achieve the objective, the strategy of design was block-type polymers composed of two segments: a cytocompatible 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer segment and a pH-responsive segment. A fluorescent dye was immobilized to the polymer-modified QDs. The pH-responsive segment provided a nice dynamic platform for the fluorescence resonance energy transfer (FRET) between the donor QD and acceptor fluorescent dye. That is the real time monitor would be performed by of the FRET properties through polymer chain movement.

The advance of the well-defined polymer design is mainly own to RAFT polymerization. The uniform design of polymer chains advanced the pH sensitivity, precise positioning of the FRET distance, free transforms of stimuli sensitive segment and biomolecular immobilization. The advanced nanoparticle systems would provide a multifunctional platform for revolutions in the polymeric fluorescent probe, biomolecular carrier and effective monitors of not only intracellular pH but also other intracellular conditions of living cells.

The dissertation is organized from 6 chapters:

Chapter 1. General Introduction

The significance, methods and advances of nanoparticles to develop biomaterials and intracellular applications were described. The well-defined polymer designed via living radical polymerization was the main modified method to prepare a polymer-based fluorescence nanoparticle. The well-defined polymer modified surfaces and nanoparticles were introduced. Intracellular pH is a profound factor of cellular function and provides information not only in the physiology but also in the progress of organelle. The traditional intracellular pH measurements and their limitation and evaluation were summarized. On this basis, a molecular design concept of polymer that satisfies the characteristics of intracellular pH nanoparticles was presented.

Chapter 2. Design of multi-functional well-defined polymers

The RAFT polymerization synthesized block-type polymers were consist of one cytocompatible MPC polymer segment and another pH-responsive poly(2-(N,N-diethylamino)ethyl methacrylate (DEAEMA)) segment with different molecular architecture: diblock polymer poly(DEAEMA-*block*-poly(MPC-*random*-*n*-butyl methacrylate (BMA)-*random*-*p*-nitrophenyloxycarbonyl oligo(ethylene glycol) methacrylate (MEONP))) (PDbMBN), and triblock polymer poly(DEAEMA-*block*-MEONP-*block*-MPC) (PDbNbM). The BMA unit provided amphiphilic nature to the polymer. The MEONP unit was introduced to polymers for biomolecules immobilization. The surface tensions of the polymer aqueous solutions decreased from the concentration about 0.01 mg/mL. The pH values was also affect to polymer aggregation movement. The different polymer architecture had effect on the surface tension measurement of polymers. The polymer chains without hydrophobic units got more freedom. The irregular size change of PDbMBN and PDbNbM at different pH values suggested that the active ester groups in the polymers produced some ions to interact the aggregation movement of polymers. The well-structured, water-soluble, pH sensitive and cytocompatible polymers were the shell of nanoparticle.

Chapter 3. Surface hybridization of QD nanoparticles with well-defined polymers

QD/well-defined polymer hybrid nanoparticles were prepared by solvent evaporation method. The modified nanoparticles got some excellent properties as expected, that is, water-dispersible, controllable sizes, optical properties and pH responsiveness. The zeta-potential of nanoparticles was positive charge around 25.1 mV~30.1 mV in the acid condition and negative charge -9.3 mV in the alkaline condition. The diameters of nanoparticles changed in various pH conditions. The zeta-potential and diameters of nanoparticles indicated that the nanoparticles was response to pH. A factor to influence the diameters of nanoparticles was polymer concentration, and sonication time had impact on weakening the optical properties of nanoparticles. Through combining the results of above parameters in the nanoparticle preparation, multifunctional nanoparticles can be prepared to improve the investigation of cellular environment by immobilized biomolecules.

Chapter 4. FRET detection in different pH condition

Chapter 4 discussed the addition function, easy immobilization of target fluorescence dye of well-defined polymer on the nanoparticle similar with a nanocarrier. The fluorescence dye Alexa was introduced to polymers via the active ester group of MEONP units. The MEONP unit in the PDbMBN polymer chain was single. There was 50 nmol MEONP units in the 1.0 mL QD/PDbMBN nanoparticle solution. This result is the reference for inferring the active ester groups in other polymer chains with different numbers of MEONP units. When the fluorescent dye Alexa was used as the FRET acceptor in

the QD/polymer hybrid nanoparticles, the FRET phenomenon was observed. The efficiency of FRET was dependent on the pH of the solution because of changes in the diameter of the hybrid QDs at different pH values caused by the stretching–shrinking conformational changes that occur upon protonation of the pH-responsive segments of the block-type polymer. Moreover, the QD/PDbNbM-Alexa nanoparticle displayed a higher FRET efficiency than others. The location of MEONP in the QD/PDbNbM-Alexa was clearly decided between the poly(DEAEMA) segment and PMPC segment. The distance from the dye to the QD was easier to be controlled than other polymer structures. The design of the molecular architecture played an important role in this multifunctional nanoparticle system. Therefore, the QD/PDbNbM-Alexa hybrid nanoparticles had the potential to evaluate the pH of cells.

Chapter 5. Evaluation of intracellular condition by QD/polymer-Alexa nanoparticles

The nanoparticles were immobilized with the cell-penetration peptide octaarginine (R8) on the terminal of polymer chains. The cytotoxic test was confirmed by amount of lactate dehydrogenase LDH released from the cell membrane. Cell membrane was less injured by R8-QD/PDbNbM-Alexa nanoparticle at low concentration. R8-QD/PDbNbM-Alexa nanoparticles successfully tracked intracellular pH in endocytosis progress by on-off FRET phenomenon. The continuous observation of the R8-QD/PDbNbM-Alexa movement provided a dynamic method for effective tracking endocytosis process. QD/PDbNbM-Alexa nanoparticles could enter cells by themselves. However, cell uptake speed and number of nanoparticles, and area of endosomes could be influenced via immobilization of R8.

Chapter 6. Conclusions and future perspective

This study created the nanoparticles for elucidating the chemical reactions occurring in the organelle level, and proposing a design concept of nanodevices. The useful information in terms of understanding and control of molecular reactions in cell engineering was provided. The good performed nanoparticles contribute significantly to the development of bioengineering.