Phosphorylcholine-Based Nanoparticles Covered with Fluorescence Resonance Energy Transfer System for Smart Bioassay

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Introduction: Phosphorylcholine (PC)-based interface shows not only suppression of non-specific protein adsorption but also stabilization of immobilized biomolecules [1-2]. Therefore, PC-covered nanoparticles (NPs) are capable of being good matrices for bioassay. Generally, enzyme-linked immunosorbent assay (ELISA) was popular protocol for bioassay. However, the ELISA contains following disadvantages, (i) complicate protocol and (ii) non-specific adsorption. In this study, we report a new methodology for bioassay. A single protocol is designed by fluorescence resonance energy transfer (FRET) on PC-NPs. The FRET mechanism is a distance-dependent interaction between the electronic excited states of two fluorescence molecules in which excitation is transferred from a donor molecule to an acceptor molecule [3]. Therefore, each fluorescence molecule was connected with antibody, and the fluorescence-labeled antibody was then immobilized onto PC-NPs. The antibody-conjugated NPs could capture a specific antigen from sample solution, then the distance between a donor and an acceptor molecule might be close by formation of antibody-antigen complex. If the FRET takes place by agglutination of NPs, fluorescence by acceptor molecule will be observed (Figure 1).

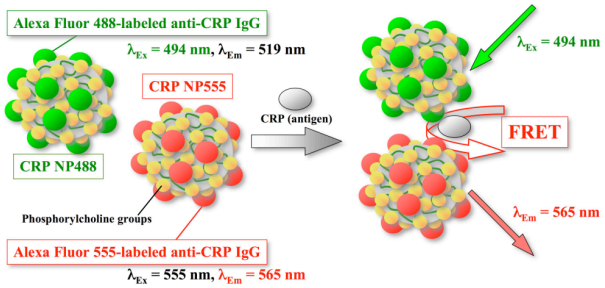

Figure 1 Bioassay using phosphorylcholine-based nanoparticles by fluorescence resonance energy transfer. Assay for C-reactive protein (CRP) was demonstrated as target molecule.

Methods: We synthesized bioconjugate PC-based polymer (PMBN) as previously reported method [4]. PMBN contains active ester groups, which are labile to the primary amino group in the biomolecules. The resulting polymer showed amphiphilic property in aqueous solution, and PC-NPs could be prepared by solvent evaporation method, and the NPs were composed of a polystyrene core with PMBN (PC) shell [5]. C-reactive protein (CRP) and osteopontin (OPN) was used as typical biomarker molecules in terms of inflammatory and differentiation markers, respectively. Furthermore, Alexa Fluor 488 (donor) and Alexa Fluor 555 (acceptor) were selected for the pair of fluorescence molecules. To obtain fluorescence-labeled antibody, each donor and acceptor molecule was conjugated with each antibody. The resulting fluorescence intensity was monitored by multi-plate reader. The agglutination of NPs was reacted in 96 well plates, and final reactive volume was 200μL in each well. Furthermore, final concentration of NPs was 10μg in each well, and a target antigen was studied at the concentration of 0.15-15μg/mL.

Results/Discussion: To obtain fluorescence-labeled antibody, each donor and acceptor molecule was conjugated with each antibody. The conjugation efficiency was calculated from the UV-Vis spectrum, and was roughly 3 mol/mol. The resulting each fluorescence molecule-labeled antibody was immobilized onto NPs, respectively. Figure 2 shows change in fluorescence intensity by CRP and OPN assay. In the reactive media, NPs covered with donor-labeled CRP antibody or acceptor-labeled CRP antibody was dispersed. If the CRP antigen was captured by NPs, NPs could form agglutination in the well. The intensity was strongly dependent on the concentration of target antigen. Therefore, the bioconjugate PC-NPs could specifically detect biomarker molecules. Fortunately, the resulting fluorescence intensity was well correlated with a concentration of target molecule, not only CRP but also OPN. This result indicated that plausible agglutination took place, when an antigen was added to the reactive wells. PC-NPs covered with FRET system are good candidate for smart bioassay.

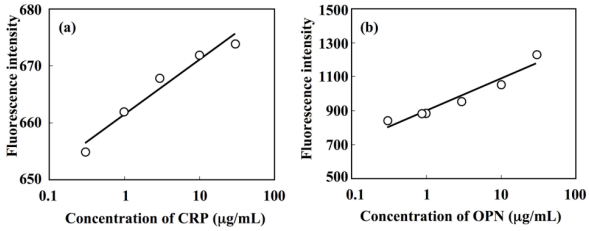

Figure 2 Change in fluorescence intensity by capture of (a) CRP and (b) OPN. Nanoparticles (10μg/well) were used.

Conclusions: We developed a smart use of FRET for bioassay. The resulting information regarding bioassay was readable by FRET system, which was installed in NPs. The diversity of target molecules was easily designed by tuning fluorescence-labeled antibody, which was tailored. Thus, it suggests that one could tailor the FRET system-installed NPs for smart bioassay.

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References: [1] Ishihara K. Sci Tech Adv Mater. 2000;1: 131-138. [2] Sakaki S, Nakabayashi N, and Ishihara K. J Biomed Mater Res. 1999;47:523-528. [3] Berney C, and Danuser G. Biophys J. 2003;84:3992-4010. [4] Konno T, Watanabe J, and Ishihara K. Biomacromolecules 2004;5:342-347. [5] Watanabe J, and Ishihara K. Biomacromolecules 2006;7:171-175.