Inhibition of Inflammatory Responses on Phospholipid Polymer-coated Nanoparticles

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Statement of Purpose: Inhibition of unfavorable biological response on the interface is most important for understanding real functions of biomolecules on the surface. One of the best ways to design the biomaterials is generated from mimicking a cell membrane structure. The surface of cell membrane-like structure is constructed artificially by molecular integration of phospholipid polymer as platform and conjugated biomolecules. Here, it is introduced the effectiveness of biointerface with highly biological functions observed on artificial cell membrane structure. Reduction of nonspecific protein adsorption is essential for suppression of unfavorable bioresponse and achieving of versatile biomedical applications. Simultaneously, bioconjugation of biomolecules on the phospholipid polymer platform are crucial for a high-performance interface. The nanoparticles covered with the artificial cell membrane will be discussed from viewpoints of prevention of bioresponses even when they are in cellular systems.

Methods: The a water-soluble polymer composed of 2-methacryloyloxyethyl phosphorylcholine (MPC), *n*-butyl methacrylate(BMA), and *p*-nitrophenyloxycarbonyl poly(oxyethylene)methacrylate (MEONP) units (the polymer is named as PMBN) was prepared by a conventional radical polymerization. The PMBN easily enriched onto the poly(lactic acid) (PLA) nanoparticle (PMBN/PLA) surface by the solvent evaporation method. Polymer nanoparticles embedding quantum dots (QDs) covered with PMBN were designed by making assemble of phosphorylcholine groups as platform and biomolecules immobilized on the surface of nanoparticles. We observed that PMBN/PLA/QD had the abilities to highly resist to nonselective cellular uptake from HeLa cells and to permeate the membrane of HeLa cells effectively when arginine octapeptide (R8) was immobilized on the surface of the nanoparticles.

Mouse macrophage RAW264.7 cell, known for its sensitivity to inflammatory response, was used as a model system. RAW264.7 cell was incubated with PMBN/PLA/QD for 24 h. After incubation, the inflammatory response was measured by using real-time reverse transcription polymerase chain reaction. Oligonucleotides TNF-α-F (5'-GAGCAGCTGGAGTGGCTGCTGAG-3') and TNF-α-R (5'-TAGACCTGCCCGGACTCCGC-3') were used for detection of tumor necrosis factor-alpha (TNF-α); GAPDH-F (5'-AATGTGTCCGTCGTGGAT CT-3') and GAPDH-R (5'-CCCTGTTGCTGTAGCCG TAT-3') were used for glyceraldehydes-3-phosphate dehydrogenase (GAPDH). The expression of TNF-α mRNA was standardized as the relative value to that obtained for GAPDH mRNA. As a negative and positive control, a normal cell and the cell with 100 ng/mL lipopolysaccharide were used respectively.

Results: The PMBN/PLA shows the bioinert abilities and they may avoid phagocytosis from macrophage-like cells. At the surface of the PMBN/PLA/QD various amino acid and their oligopeptide could be immobilized. The glycine-immobilized PMBN/PLA/QD (glycine-PMBN/PLA/QD) completely suppress the nonselective uptake from HeLa cells. We also confirmed that no glycine-PMBN/PLA/QD was uptaken by HeLa cell even after incubation for 24 h. In general, conventional nanoparticles can be uptaken by the cells without any selectively. Thus, this result indicated that our PMBN/PLA/QD can obtain high signal to noise ratio compared with other imaging probes because there is no background fluorescence caused by nonselective uptake of imaging probes. On the other hand, R8-conjugated PMBN/PLA/QD internalized effectively into cells. The uptake of R8-PMBN/PLA/QD significantly increased within first 15 min, but the uptake rate gradually slowed and reached a plateau at 1 h. The PMBN/PLA/QD have no cytotoxicity for 3 days even after internalization in HeLa cells. However, there are some possibilities of the induction of inflammation reaction even when no cytotoxicity appears. Thus, more studies are required with respect to the inflammatory response. Fig. 1 shows the relative expression of TNF-α mRNA to GAPDH mRNA in RAW264.7 cells incubated with R8 or glycine-PMBN/PLA/QD for a day. No significant difference between negative control and PMBN/PLA/QDs in the expression of TNF-α mRNA was observed. This indicated that phosphorylcholine groups on the surface of PMBN/PLA/QD suppress the inflammatory reaction in RAW264.7 cells even when internalizing into cells due to the ologopeptide. From these findings, our PMBN/PLA/QD can eliminate the unwished interactions between probes themselves and cell, such as a nonselective cellular uptake, cytotoxicity and inflammation response.

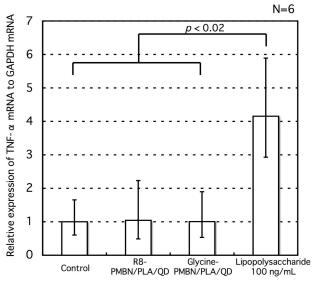

Fig. 1. Relative expression of TNF-α mRNA to GAPDH mRNA in cells incubated with polymer nanoparticles.

Conclusions: Phospholipid polymers are effective to inhibit inflammatory response of nanoparticles.

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