Characterization of Temperature-Responsive Liposome with Tunable Surface Property

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ABSTRACT SUMMARY
We prepared poly (N-isopropylacrylamide-co-N, N–dimethylaminopropyl acrylamide) (PNIPAAm-co-DMAPAAm)-modified liposomes and their temperature-dependent behaviors were investigated. We examined the fixed aqueous layer thickness (FALT) of polymer-modified liposome, and elucidated the connection between physical character and biological properties of these liposomes. Above the lower critical solution temperature (LCST) of the copolymers, the FALT of the liposomes was decreased and the enhancement of the drug release from the liposomes was observed. The contents release behavior, surface properties, and cell affinity of these liposomes can be controlled in a temperature-dependent manner.

INTRODUCTION
Liposomes are recognized as useful drug carriers, but have some problems to overcome. Liposomes are easily opsonized with serum proteins (opsonization) and taken up by the reticuloendothelial system (RES) cells, such as spleen and liver. Polyethylene glycol (PEG) modification on the liposomal membrane forms a fixed aqueous layer and thus prevents opsonization and uptake by the RES.

We have been investigated the drug delivery system utilizing functional polymer-modified nanoparticles\textsuperscript{1}\textsuperscript{3}. Poly (N-isopropylacrylamide) (PNIPAAm) undergoes a sharp phase separation and coil-globule transition of polymer chain in water across LCST at 32 °C. The polymer chains show an expanded conformation in water below the LCST due to strong hydration, and change to compact forms above the LCST by dehydration. The LCST can be tuned to a desired temperature range by copolymerization with a more hydrophilic comonomer which raises the LCST, or a more hydrophobic comonomer which lowers the LCST. Using these features, we prepared temperature sensitive liposomes modified with PNIPAAm-co-DMAPAAm.

In this study, for physicochemical characterization, the particle size, zeta potential, and the FALT were measured. The ability of the polymer coating to prevent the adsorption of human serum albumin (HSA) was evaluated, and the cellular uptake of the PNIPAAm copolymer-modified liposome was assessed. Moreover, temperature-dependent release behaviors were investigated, and elucidated the connection between physical character and biological properties of these liposomes.

EXPERIMENTAL METHODS
PNIPAAm-co-DMAPAAm was grafted to the dioleoylphosphatidylethanolamine (DOPE). The immobilization reaction involved the formation of an amide bond between the carboxylic acid end group of copolymer and amino groups of DOPE, using activated ester coupling. The liposomes were prepared from mixtures of DOPE, Egg phosphatidylcholine (PC) or 1, 2-dioleyl-3-trimethylammonium propane (DOTAP) and DOPE–grafted copolymer. The liposome suspension was extruded through polycarbonate membrane filters with 0.1 μm pores, to obtain a homogeneously-sized liposome suspension. Particle size and FALT were determined using dynamic light scattering (DLS) as a function of electrolyte concentration.

The cellular uptake of RAW264.7 cells was observed using carboxyfluorescein (CF) encapsulated in the polymer-modified liposomes by fluorescence microscope. To investigate the therapeutic efficacy, irinotecan (CPT-11) was encapsulated into the PNIPAAm copolymer-modified liposomes, and the \textit{in vitro} anticancer
RESULTS AND DISCUSSION

The LCST of PNIPAAm-co-DMAPAAm was 41°C. Size of the liposomes was approximately 150 nm. Incorporation lipid-grafted PNIPAAm-co-DMAPAAm into liposomal membranes enhanced the stabilities of liposomes in HSA by inhibiting protein adsorption.

The FALT of PNIPAAm copolymer-modified liposome was decreased above the LCST, compared with that of below the LCST, as shown in Fig.2. On the other hand, the enhancement of the cellular uptake was observed above the LCST of the copolymers.

The liposomes hardly released the contents below the LCST of the copolymer, but the release was drastically enhanced above that temperature. It was indicated that the drug release was triggered by alteration of the polymer chain of liposome surface from a hydrophilic state to a hydrophobic state occurring at their LCST. While the copolymer-modified liposomes released little below 37°C, release was enhanced above 42 °C, indicating that dehydrated copolymer chains destabilized the liposome membrane. Furthermore, to investigate the therapeutic efficacy, CPT-11 was encapsulated into the temperature-sensitive liposomes, and the in vitro anticancer activity of was evaluated using human cancer cell lines, Caco-2 and HEK293. Fig.2 shows in vitro anticancer activities of temperature-sensitive liposomes using Caco-2 cells. The cellular survival rate was measured by WST-1 assay. The percentage of survival rate was significantly lower in the polymer modified liposome at 42 °C than in unmodified liposome (p < 0.001). As the results, these functional liposomes which contents release behavior, surface properties, and affinity to cell surface can be controlled in a temperature-dependent manner. The release of anticancer drugs from the copolymer-modified liposomes can be controlled by temperature changes, which alter interactions between polymer chains and liposomal membranes.

CONCLUSION

Our research indicates clearly that the value of the FALT around the liposome, formed by PNIPAAm-co-DMAPAAm modification, is tunable by a changing temperature and correlates with the cellular uptake. Moreover, the drug release from liposome can be controlled by temperature changes. The temperature-responsive liposome will be efficacious carriers for the delivery of anticancer drugs, and have anticancer applications in combination with hyperthermia.

REFERENCES