Plasmonic Liposomes for Synergistic Photodynamic and Photothermal Therapy

Jeongmin Oh¹, Hwan-Jun Yoon¹ and Ji-Ho Park¹

¹Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Republic of Korea
jihopark@kaist.ac.kr

ABSTRACT SUMMARY
Plasmonic liposomes (PL) were developed to carry hydrophobic photosensitizers (zinc phthalocyanine, ZnPc) with high unimer form and perform dual phototherapy using a single NIR light source. ZnPc-loaded PL appeared fairly spherical with sizes in the range of 80-120 nm and an absorption wavelength of 670 nm. Photodynamic effect of ZnPc-loaded PL can be dramatically enhanced when combined with their photothermal effect.

INTRODUCTION
Phototherapy is a promising strategy for cancer treatment due to its selective and localized therapeutic effect by laser irradiation¹,². There are two major types of phototherapy: photothermal therapy (PTT) and photodynamic therapy (PDT). PTT damages malignant cells with heat converted from light by photoactive agents. For the PTT, biocompatible gold nanoparticles have been widely studied as potential agents as they showed efficient conversion of absorbed light into heat. On the other hand, PDT uses photosensitizers that become cytotoxic upon laser irradiation at their excitation wavelength. For the PDT, zinc phthalocyanine (ZnPc) has been widely used as a photosensitizer because it is highly active at near-infrared (NIR) wavelength. However, due to its hydrophobicity, ZnPC must be encapsulated into nanocarriers for biological applications. Among such nanocarriers, clinically approved liposomes are known to load photosensitizers into their transmembrane with high unimer form³.

As singular treatment of each phototherapy has showed some limitations, there have been significant efforts to combine these two phototherapies. Previous studies have mostly focused on dual phototherapy systems with two different laser sources⁴,⁵. However, there has been little effort to engineer a biocompatible phototherapeutic nanocarrier that is capable for dual phototherapy with a single light source. Here, we present plasmonic liposomes that can carry hydrophobic photosensitizers with high unimer form and of which the intrinsic NIR absorption enables synergistic photodynamic and photothermal therapy of cancer.

EXPERIMENTAL METHODS
ZnPc-loaded liposomes (ZnPc-L) were prepared from HSPC, DSPE-NH2, and ZnPc in the molar ratio of 69:6:2. Briefly, after mixed, the solvent is removed to yield a lipid film. Hydration of the dry lipid film is accomplished simply by adding DI water to the vial of dry lipid and stirring at 60 °C. After hydration, the liposomes were extruded through a polycarbonate filter with 100 nm pore size. Hydrodynamic size of the liposomes was measured by dynamic light scattering.

To prepare ZnPc-loaded plasmonic liposomes (ZnPc-PL) that have an absorption (plasmonic) peak at 670 nm, which is the absorption peak of ZnPc for photodynamic therapy, a gold coating on the liposome was done using published deposition-precipitation process with slight modifications⁶. Gold ion solution was prepared with 2.4 ml of DI water, 0.3 ml of 1% HAuCl₄·3H₂O, and 36 µl of 1N NaOH. This mixed solution was aged for more than 1 h at room temperature. 180 µl of the gold ion solution and 320 µl of the liposome solution were mixed for 10 min and then the mixed solution was added with 70 µl of 20mM NH₂OH·HCl as a reductant. After reduction for 5 min, their size and morphology were observed with transmission electron microscopy (TEM). Their absorption and fluorescence spectrum were analyzed by UV-vis and fluorescent spectrometry, respectively.

To test photodynamic (PD) effect of ZnPc-PL, the amount of reactive oxygen species from the sample (containing ZnPc-loaded PL) was measured using singlet oxygen sensor green (SOSG), which shows destructive change to fluorescence form when exposed to singlet oxygen. 5 µl of SOSG and 380 µl of sample solution were mixed in the well of 96-well plate. The mixed solution was then irradiated for 5 min using a 660 nm laser source (1.63 mW/mm²). After irradiation, the nanoparticles were removed by centrifugation to prevent any quenching effect on the sensor fluorescence. The green fluorescence of the sample, derived from exposure to singlet oxygen, was analyzed using fluorescence spectrometry. The value of green fluorescence can be a quantitative indicator of the PD effect. To test photothermal (PT) effect of ZnPc-PL, temperature change of the samples upon irradiation was real-time monitored using a thermo-IR camera.

RESULTS AND DISCUSSION
Plasmonic liposomes that absorb light at the NIR wavelength where ZnPc is excited for PDT were prepared by coating gold nano-film on the liposome surface using the deposition-precipitation process. ZnPc-PL were well dispersed in an aqueous solution. In TEM, they appeared fairly spherical with a thin gold coating over liposome template and sizes in the range of 80-120 nm.

ZnPc-PL showed distinct absorption peak at 670 nm which is the excitation wavelength of ZnPc for PDT (Figure 1A). Although the absorption peak of loaded ZnPc was overwhelmed by that of PL, fluorescence peak of loaded ZnPc was clearly observed with a ZnPc-PL sample (Figure 1B). The relatively low fluorescence intensity of ZnPc loaded in the PL compared with that of
ZnPc loaded in the liposome is attributed to partial quenching of ZnPc fluorescence on the gold surface.

![Absorption and fluorescence spectra of ZnPc-L and ZnPc-PL](image)

**Figure 1.** Absorption (A) and fluorescence (B) spectra of ZnPc-L and ZnPc-PL. Both liposomes contain the same amount of ZnPc.

Next, we simultaneously tested photothermal and photodynamic effect of ZnPc-PL. ZnPc-PL samples at different particle concentrations were prepared based on ZnPc concentrations and placed in each wells of 96-well microplate with the fluorescent sensor of singlet oxygen. Each well was then irradiated with a 660 nm laser for 5 min. The temperature change of samples was monitored during the irradiation and the fluorescence change of samples was measured after the irradiation.

![Photothermal effect](image)

**Figure 2.** (A) Photothermal effect of ZnPc-PL upon irradiation. Base line of the temperature was room temperature. (B) Photodynamic (PD) effect of ZnPc-PL upon irradiation. Fluorescence signal from ROS sensor was used to detect PD effect. Error bars indicate standard deviations of three parallel samples. Statistical analyses were performed with independent-samples t test (*p < 0.05).

In PT experiments, ZnPc-PL at the highest concentration (10 µM ZnPc) exhibited the temperature increase up to 20 °C upon 5-min irradiation. Their PT effect is similar to that of PL alone at the same gold concentration, indicating that the ZnPc loaded in the liposomal transmembrane did not hamper the coating of gold nanostructure that is responsible for PT effect. PT effect of ZnPc-PL was increased with increase of their particle concentrations. Liposome alone and ZnPc-L did not show any significant PT effect. Thus, the PT effect of ZnPc-PL is mainly attributed to the gold nanostructure coated on the liposome surface that efficiently converts absorbed NIR light to heat.

In PD experiments, sensor fluorescence of ZnPc-L samples was increased with increase of their particle concentration. Interestingly, PD effect of ZnPc-PL is significantly higher than that of ZnPc-L at a ZnPc concentration of 1 µM while similar to that of ZnPc-L at a concentration of 10 µM. However, ZnPc-PL at a ZnPc concentration of 10 µM exhibited much lower PD effect than them at a ZnPc concentration of 1 µM. We postulate that ZnPc loaded in the PL was not fully activated for PD effect upon light irradiation because gold nanostructure of the PL absorbs most of light at high particle concentration. These results suggest that PD effect of ZnPc-PL at an appropriate concentration can be dramatically enhanced using a single light source when combined with their PT effect.

**CONCLUSION**

In this study, plasmonic liposomes were developed to carry photosentizers ZnPc with high unimer form and perform dual phototherapy using a single NIR light source. We demonstrated that photodynamic effect of ZnPc-loaded plasmonic liposomes can be dramatically enhanced when combined with their photothermal effect. We believe that this phototherapeutic liposome has great potential to improve current phototherapy of cancer.

**REFERENCES**


**ACKNOWLEDGMENTS**

This research was supported by the National R&D Program for Cancer Control, Ministry for Health and Welfare, Republic of Korea, through grant no. 1220070.