약학석사학위논문

Photoresponsive Liposomes for Anticancer Photothermal Therapy

광감응 리포좀을 이용한 광열 항암 치료

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서울대학교 약학대학원

약학과 물리약학

황 지 현

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이 논문을 약학석사학위논문으로 제출함

2015년 12월

서울대학교 대학원

약학과 물리약학전공

황 지 현

황지현의 석사학위논문을 인준함

2015년 12월

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Abstract

Photoresponsive Liposomes for Anticancer Photothermal Therapy

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This thesis discusses the delivery of photo-responsive liposomes, lipid-based nanoparticles, to cancer cells for an anticancer photothermal effect with simple near-infrared light radiation. Dopamine (DA) is a catecholamine neurotransmitter hormone (153 Da) that may be oxidized and self-polymerized into polydopamine (PDA) under basic conditions, in this case pH of 8.5. From the previous studies, PDA has strong adhesive properties towards solids of any chemical composition through covalent or non-covalent interactions, therefore, it was used for bio-inspired surface modifications on liposomes, which also brought its photo-responsive nature to NIR light at 808nm at the same time; this type of adhesive coating method was selectively derived from the adhesive mechanism of the mussels and is widely used for its

biocompatibility. The experimental results showed various promising effects. First the temperature of modified photo-responsive liposomes increased about 69 ^{o}C (at maximum) above that of the control group under the NIR light, which in turn brought photo-thermal effect of the cancer cells. The results of in vitro efficacy tests of the modified liposomes showed low cell viability compared to other two groups - untreated and normal liposomes. Lastly, the liposomal delivery of Edelfosine - one of the most common anti-cancer drugs - reduced viability of the cancer cells remarkably after PTT. Overall, this development of the photo-responsive liposomes, encapsulating anticancer drug, suggests combinatorial chemo- and photothermal- therapy as a new approach to the development of future anticancer medicines.

Key words: Liposomes, Near infrared light, Bio-inspired surface modification, Biocompatibility, Photothermal therapy, Chemotherapy

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Ⅰ. Introduction

Photothermal therapy (PTT), one of the recently developed cancer treatments, has been considered as an alternative method for nonsurgical cancer therapy [1]. PTT has been used to treat tumor cells both selectively and remotely through irradiation at a specific wavelength of light [2]. On the other hand, chemotherapy results systemic side effects due to its unspecific drug delivery to all tissues including normal and abnormal ones [3-5]. Moreover, cancer chemo- treatment may results in drug resistance [6]; to overcome its side effects, PTT can be promising assistant cancer treatment for optimized efficacy.

Generally, gold, iron oxide, carbon and other inorganic nanomaterials are used as photothermal nanoparticles responsive to near-infrared (NIR) light between wavelength of 700 and 1100nm [7-8]. Photoresponsive materials abosorb NIR light and they go under conversion process from light to heat [3,9]. With biocompatibility and capability of high performance as a PTT agent, PDA are explored as photo-absorbers [10] for anticancer therapy. Although PDA itself provides a high capacity of anticancer photothermal effect, it may not maximize the therapeutic efficacy; therefore, the combination of chemo and photothermal therapy is a useful strategy for optimizing efficacy and minimizing the dosage-related side effects in the treatment of solid tumors [11].

In this study, we explored the use of photoresponsive and heat-producing, material, PDA, a mimic of mussel adhesive proteins, as a substance for surface modification on nanoparticles [12]. In addition, the anticancer drug of

edelfosine as a chemotherapeutic agent was encapsulated in nanoparticles. Therefore, our new photothermal multifunctional nanoparticles for cancer thermo-chemo therapy will be discussed in the thesis.

Ⅱ. Materials & Methods

2. 1. Cell culture

The BT-474 (human breast carcinoma) cells were maintained in Rosewell Park Memorial Institute Medium (Gibco BRL life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 units/ml of penicillin, 100 μg/ml of streptomycin (complete RPMI media). The A375 (human melanoma) cells were maintained in Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 units/ml of penicillin, 100 μg/ml of streptomycin (complete DMEM media). Both cells were placed in 5% CO_2 and 95% oxygen at 37°C in a humidified incubator.

2. 2. Preparation of PDA.

Dopamine hydrochloride (10mg/ml ; SigmaAldrich) was dissolved in 10mM Tris-HCl (pH8.5). PDA was prepared by oxidizing dopamine solution for an hour at room temperature. It can self-polymerize at basic pH 8.5 buffer solution, and the coating was performed with this simple mixing method with a ratio of 1:1 (PDA:liposome). PDA can spontaneously deposit on liposomes' surface form a conformal layer.

2. 3. Preparation of liposomes

Liposomes were prepared by the previous described method modifying the composition and ratio of lipids. To prepare anionic liposomes, egg-L-α-phosphatidyl-DL-glycerol (PG; Avanti Lipids, Birmingham, AL, USA) and cholesterol (Chol; Avanti Lipids) were mixed at a molar ratio of 10:5 (PG:Chol) with total 15 μmole of lipids. To prepare edelfosine (Tocris Bioscience, Bristol, UK) encapsulated liposomes, 19mM of edelfosine in chloroform was combined with the mixed lipids at a molar ratio of 10:5:5 (PG:Chol:Edelfosine). To prepare fluorescent liposomes, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (triethylammonium salt) (NBD-PE, Invitrogen, Carlsbad, CA, USA) was used by simply adding 6.2 μmole of the described lipid mixtures. After all the lipids were mixed, then all organic solvents must be removed in order to make thin lipid films. It hydrated with Tris-buffer at pH 8.5 and extruded (Northern Lipids, British Columbia, Canada) three times through 0.2 μm polycarbonate membrane filters (Millipore, Bdford, MA, USA).

2. 4. Characterization of the photoresponsive liposomes

To characterize the photoresponsive liposomes, ELS-Z instrument (Photal, Osaka, Japan) was used for size and zeta potential measurement. The sizes of liposomes and photoresponsive liposomes were determined using dynamic light scattering (DLS). The samples were diluted with Tris buffered and placed in ELS-Z. Zeta potential values of liposomes and photoresponsive liposomes were determined by laser Doppler microelectrophoresis at an angle of 22^{o}C using ELS-Z.

2. 5. Laser irradiation and photothermal imaging

Liposomes and PDA/liposomes were irradiated using an 808 nm continuous wave NIR diode laser beam (BWT Beijing LTD, Beijing, China) with an output power of 1.5W. The temperature and photothermal images of the liposomes and PDA coated liposomes suspensions during laser irradiation were recorded using an infrared thermal imaging system every 30 seconds (FLIR T420, FLIR Systems Inc., Danderyd, Sweden).

2. 6. *In vitro* cellular uptake study

The cellular uptakes of liposomes or PDA/liposomes were determined using confocal microscopy. The cellular uptake was visualized by labeling the liposomes with NBD-PE lipids. BT-474 human breast carcinoma cells were seeded onto 24-well plates at a density of 1.5×10^7 cell per well. When the cells reached 70% confluence, NBD-PE-labeled liposomes or PDA/liposomes were added to each well. After 2h, the cells were washed by cold phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 15 min, and stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). The fluorescence of the cells was observed using a confocal laser scanning microscope (LSM 5 Exciter; Carl Zeiss, Inc., Jena, Germany). Flow cytometry measurements were conducted by harvesting the cells and washing twice with cold PBS containing 2% fetal bovine serum. The cells were analyzed using a BD FACSCalibur flow cytometer using the Cell Quest Pro software (BD Bioscience, San Jose, CA, USA).

2. 7. Quantitative cell viability assay following NIR laser irradiation

A375 human melanoma cells were seeded onto 24-well plates at a density of 1×10^5 cells per well. The following day, cells were untreated or treated with liposomes, PDA/liposomes, edelfosine encapusulating liposomes, or PDA/edelfosine encapsulating liposomes at a edelfosine concentration of 5μM. After 2h incubation at 37°C, the cells were washed twice with cold PBS and re-suspended in complete DMEM media. Divide each group of the cells into two for distinguish between at condition with or without NIR irradiation. The cell suspensions were irradiated with an 808nm continuous-wave NIR diode laser at an output power of 1.5 W for five minutes. Immediately after irradiation, the cells were diluted 20-fold using complete DMEM media and seeded to 48-well plates for 24h. The next day, the cell viability was quantified using a 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazoli
-um bromide (MTT) assay (Sigma-aldrich, St. Louis, MO, USA). MTT solution (500 μM) was added and plate was incubated for an additional 1 h. The resulting crystal were dissoleved in 100 μl of 0.06 N HCl in isopropanol, and the absorbance was measured at 570 nm using a microplate reader (Sunrise Basic; TECAM, Männedorf, Switzerland). The values are expressed as a percentage of the cell viability measured in the control groups.

2. 8. Statistics

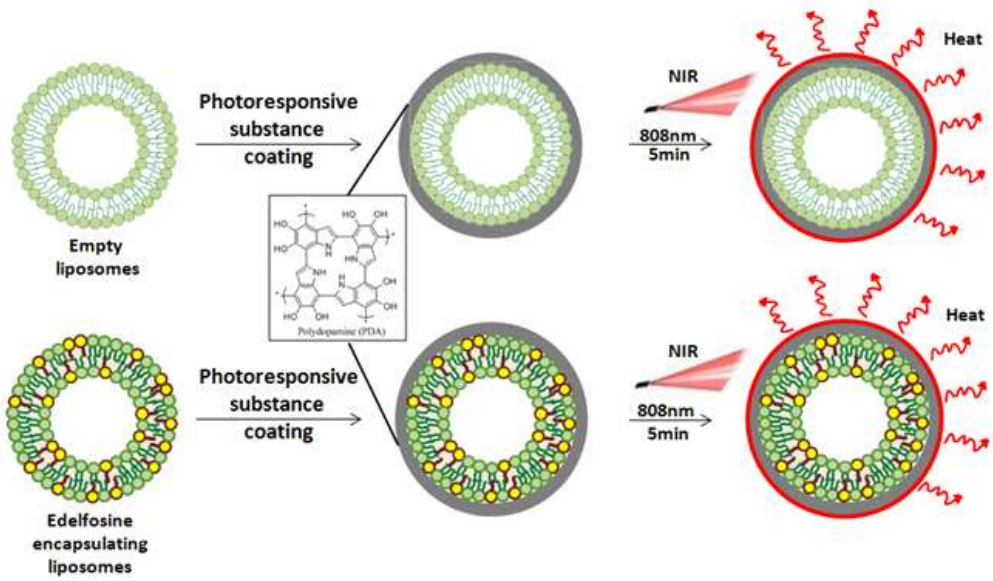
ANOVA techniques were used to statistically evaluate the experimental data. The Student-Newman-Keuls test was used as a post-hoc test. All statistical analyses were performed using the SigmaStat software (version 3.5, Systat Software, Richmond, CA, USA), and a p-value of < 0.05 was considered significant.

Ⅲ. Results

3. 1. Synthesis and characterization of PDA

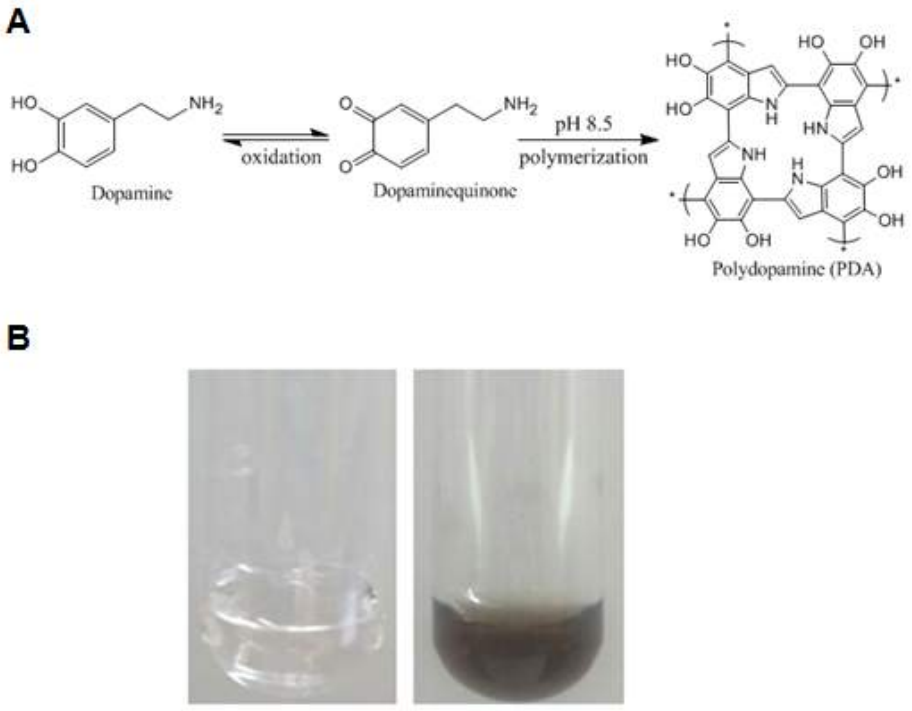
The deposition of PDA on liposomes was prepared for photothermal effects (Fig. 1) by self-polymerization of dopamine via oxidation in 10mM Tris buffer at pH 8.5 for an hour (Fig. 2A). The color of dopamine solution (10mg/ml) turned into clear first, whereas the color of PDA became dark-black after self-polymerization (Fig. 2B). The dynamic light scattering (DLS) data showed that diameters of the liposomes increased after the PDA coating (Fig. 3B). Also, the surface charges of the liposomes were changed after the PDA coating (Fig. 3C). Moreover, the UV-vis spectra showed that the PDA-coated liposomes had a higher NIR absorption rate compared to that of the non-modified liposomes (Fig. 3A).

Figure 1. Schematic illustration.



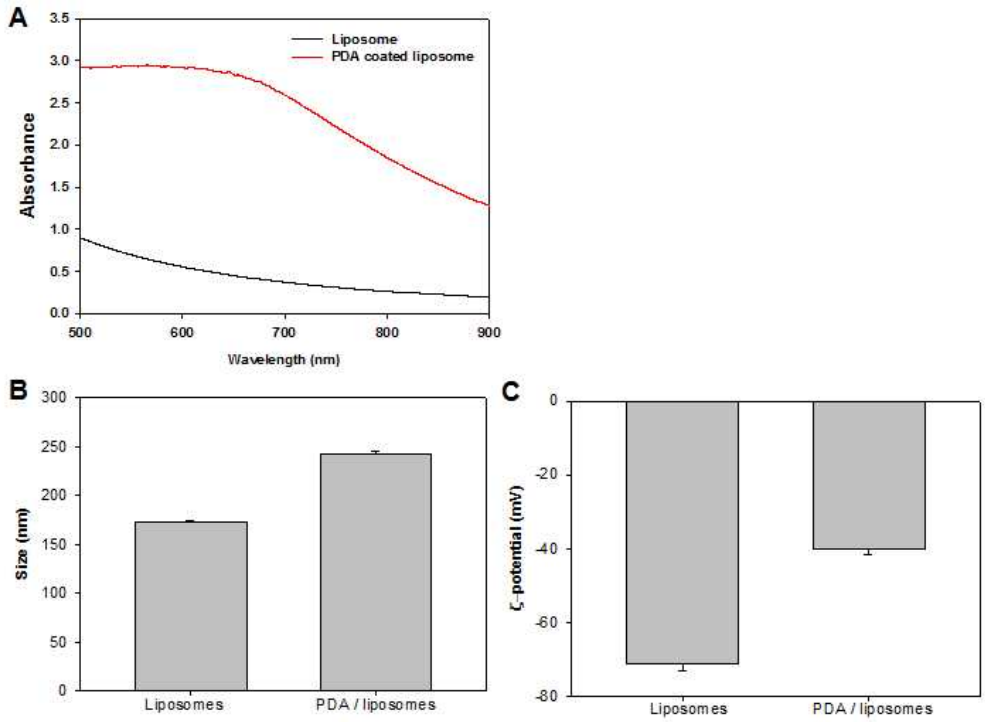
Schematic illustration of the preparation PDA coated liposomes for PTT.

Figure 2. Synthesis schemes of the PDA.



Reaction mechanism for dopamine polymerization into PDA (A) was only take an hour. The photographs of dopamine solution (concentration of 10 mg/ml) oxidized into PDA in Tris buffered at pH 8.5 (B).

Figure 3. Characterization of photoresponsive liposomes.

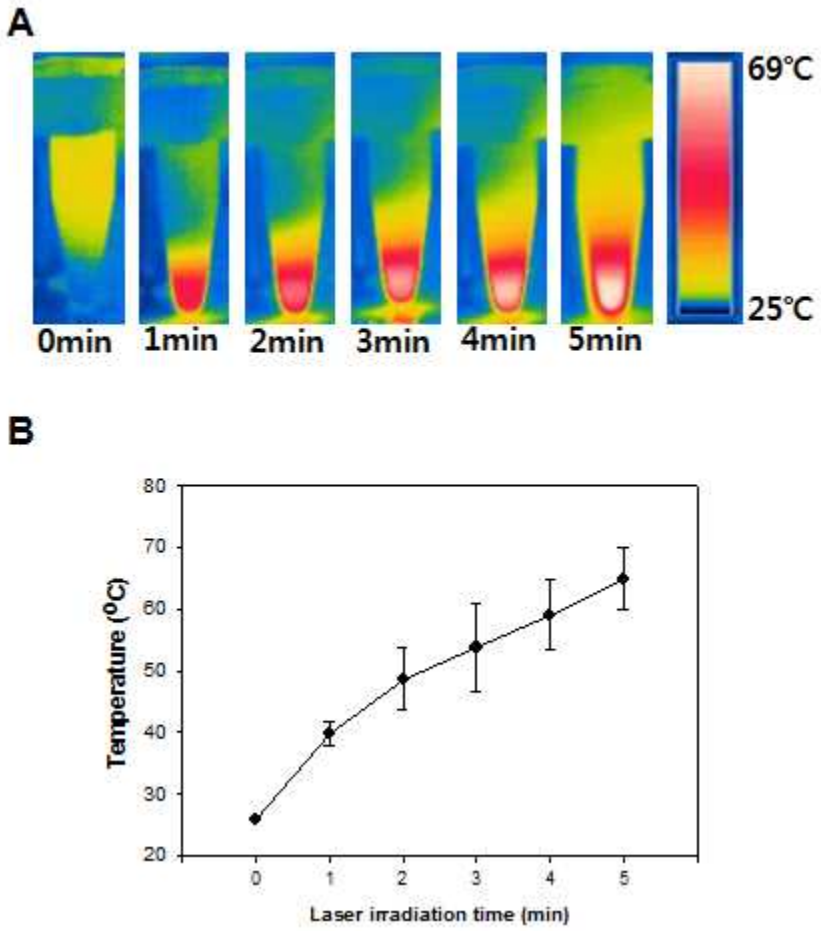


UV-vis absorbance spectra (A) was measured by UV spectrometer. The sizes (B) and zeta potential values (C) of liposomes and PDA-liposomes were measured by dynamic light-scattering.

3. 2. Photothermal capacities of PDA

The photothermal capacity was determined through real-time infrared thermal imaging of irradiated the PDA solution (10mg/ml) in 10mM Tris-buffer at pH 8.5 (Fig. 4A). The temperature of the PDA was observed in relation to the irradiation time of NIR laser (808nm). After five minutes of irradiation, the temperature of the PDA reached 64.9 ± 4.5 ^{o}C (Fig. 4B).

Figure 4. Photothermal capacities of the PDA.

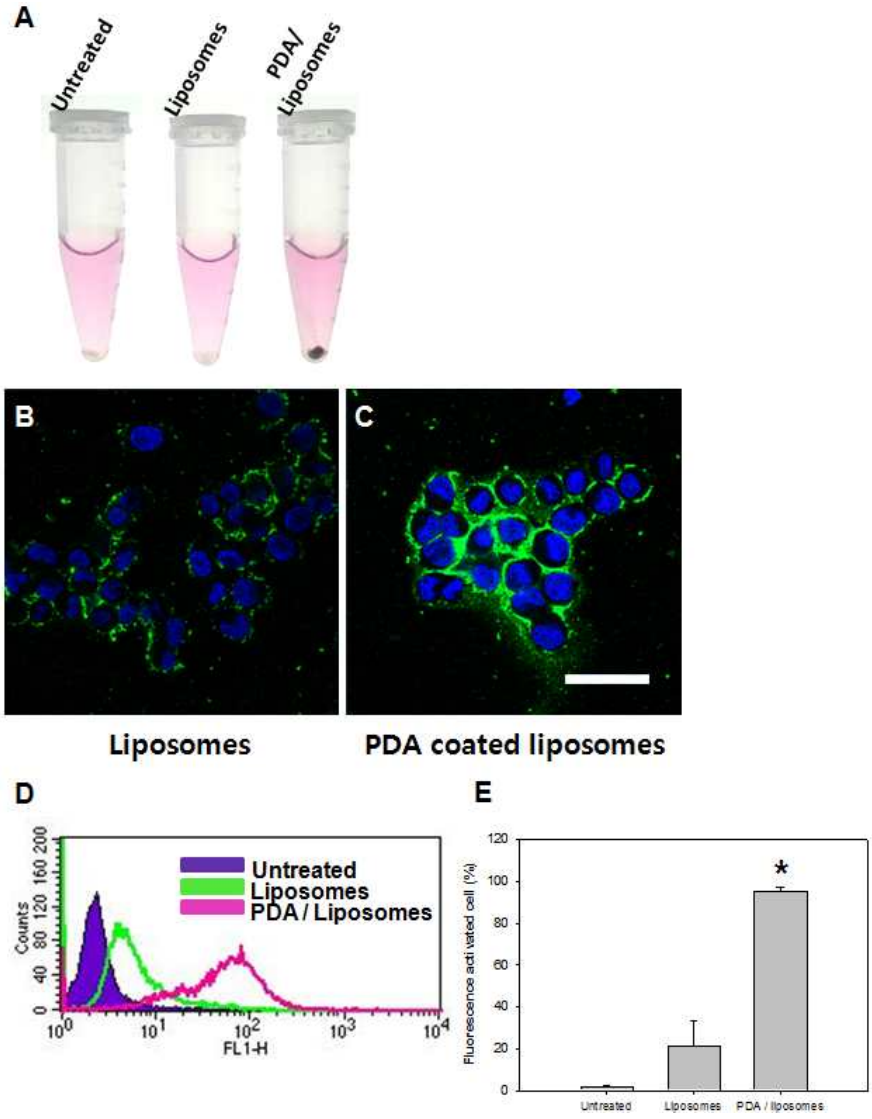


Upon irradiation with 808nm laser, the temperature of PDA (conc. 10mg/ml) in Tris buffered at pH 8.5 was observed through a real-time infrared thermal imaging techniques (A). The temperature of PDA was measured using the FLIR QuickReport 1.2 software (B).

3. 3. Cellular uptake of photoresponsive liposomes

BT-474 cells took up PDA-liposomes to a greater degree than they did for conventional liposomes (Fig. 5). As shown in (Fig. 5A), two groups of harvested cell pellets - one treated with conventional liposomes and the other with PDA-liposomes - displayed clearly different colors, white and black respectively. These qualitative observations indicated effective cellular uptake of photoresonsive liposomes for photothermal activity, which was quantitatively measured by using NBD-PE, a fluorescent dye. As observed, fluorescence confocal microscopy images revealed higher fluorescence intensity in the cells with just dye-labeled liposomes (Fig. 5C) than the cells treated with NBD-PE-labeled liposomes (Fig. 5B). Also, flow cytometry measurements of the cells revealed a 4.7-fold higher fluorescence intensity of the NBD-PE signal from the NBD-PE labeled liposomes (Fig. 5D, 5E).

Figure 5. In vitro cellular uptake of the liposomes and photoresponsive liposomes.



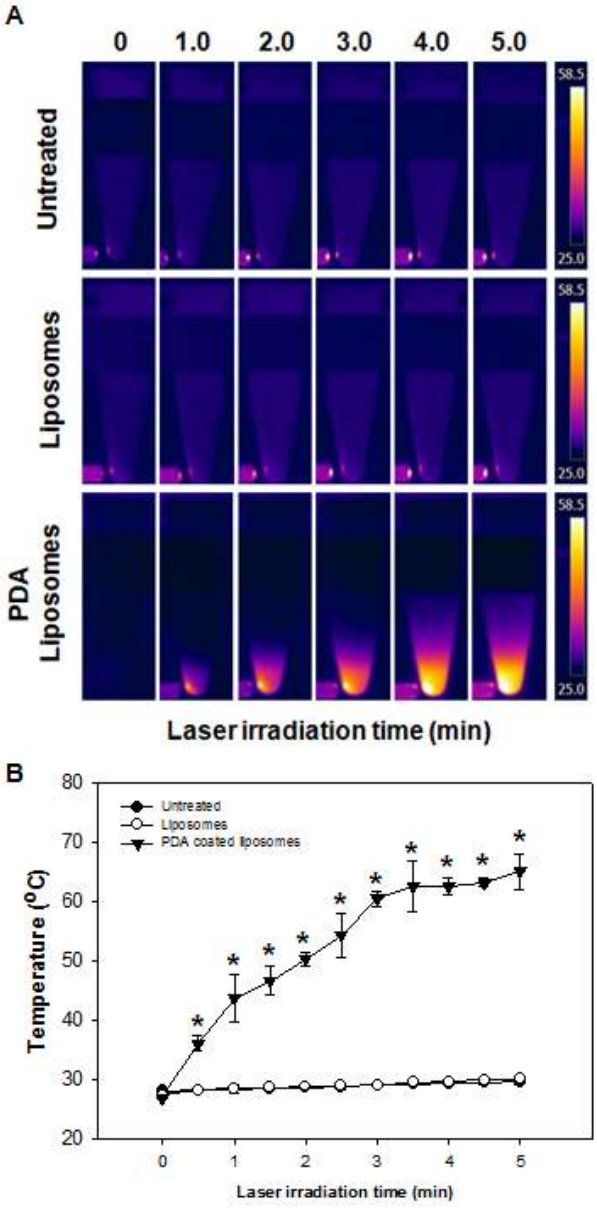
BT-474 cells were left untreated or treated with liposomes or PDA-liposomes. After 2h, the appearances of the pellets formed from untreated,

liposomes, or PDA/liposomes-treated cells were photographed (A). Fluorescence confocal microscopy images of the BT-474 cells treated with NBD-PE labeled liposomes (B) or PDA coated NBD-PE labeled liposomes (C). Representative flow cytometry data (D), and the quantitation of the fluorescence cellular intensity data (E) are presented. The scale bar indicates 20μm. *Significantly higher ($p < 0.05$) compared to the other groups (assessed by the ANOVA and the Student-Newman-Keuls test).

3. 4. Photoresponsive liposomes effect on in vitro NIR laser-induced photothermal activity

The photothermal effects produced from the irradiation of liposomes and PDA-liposomes in the treated cells were examined through real-time infrared thermal imaging (Fig. 6A). The temperature of the cell suspension was observed to increase during the five minute NIR laser (808nm) irradiation (Fig. 6A). The untreated cells showed an increase of 2.1 ^{o}C with the same irradiation time (Fig. 6B). The temperature of the cells treated with liposomes increased with the irradiation time to the same extent as did the temperature of the untreated cells. The cells treated with liposomes showed a temperature increase of 2.5 ^{o}C after five minutes of irradiation. By contrast, the cells treated with PDA-liposomes increased in temperature by 37.3 ^{o}C after five minutes of irradiation as well(Fig. 6B).

Figure 6. Photothermal effects of the photoresponsive liposomes.



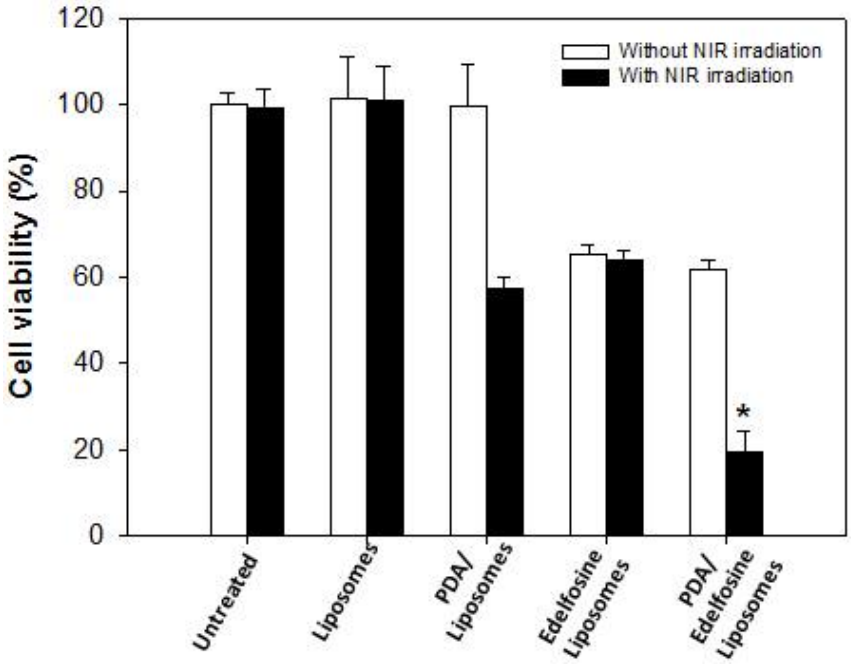
Temperature increases induced by the presence of liposomes or PDA-liposomes treated cells upon irradiation were observed using real-time

infrared thermal imaging techniques (A). The temperature of cell suspension was measured using the FLIR QuickReport 1.2 software (B). *Significantly higher ($p < 0.05$) compared to the other groups (assessed by the ANOVA and the Student-Newman_Keuls test).

3. 5. Anticancer drug encapsulated photoresponsive liposomes effect on in vitro NIR laser-induced photothermal activity

NIR laser-induced anticancer photothermal and/or chemo effects of the PDA and Edelfosine were measured using the viability test of the A375 human melanoma carcinoma cells. The survival of the tumor cells after NIR laser irradiation was found to be dependent upon the existence of PDA or Edelfosine drug in the cells (Fig. 7). The irradiation of cells with a NIR laser over five minutes did not affect the viability of the untreated cells. As a result, the photothermal effect of the NIR irradiation was shown to be effective only on the liposome groups that were modified with PDA. The liposomes that consisted of both Edelfosine and PDA showed significantly lower cell viability due to chemotherapy and PTT effects.

Figure 7. Combined photothermal-chemo cancer cell-killing effects of the anticancer drug Edelfosine-encapsulated photoresponsive liposomes.



A375 cells were left untreated or treated with liposomes, PDA-liposomes, Edelfosine encapsulated liposomes and PDA-Edelfosine encapsulated liposomes. The viabilities of the cells were measured using the MTT assay with and without laser irradiation for five minutes and then incubation for 24 hr. *Significantly higher ($p < 0.05$) compared to the other groups (assessed by the ANOVA and the Student-Newman_Keuls test).

Ⅳ. Discussion

In this study, we demonstrated that combinatorial therapy of photothermal and chemo using bio-inspired surface modification materials and anticancer drug has higher cancer cell killing potency than single therapy [11].

The PDA, which was the key part of our system was easily synthesized and modified on all kinds of organic and inorganic surfaces in aqueous state [10,12-15]. The photoresponsive liposomes were prepared by simple and versatile coating method with a uniform PDA film by dispersing in basic condition of dopamine solution at room temperature for an hour [15]. Importantly, PDA - mussel-inspired adhesion proteins - acts as an excellent redox mediator [16] and as a nontoxic [17,18] surface modification material, which makes its self-polymerization process harmless [18]. Also, dopamine contains catechol and amine groups; self-polymerization of dopamine proceeds via oxidation of catechol into dopaminequinone followed by oxidative oligomerization and further self-assembly [19]. After all, PDA can be easily distinguished from dopamine solution by color change (Fig. 2B), white and black, respectively.

The photothermal capacity of PDA was initially confirmed by UV absorbance spectra (Fig. 3A). The PDA-liposomes displayed a significantly higher NIR absorption compared to the non-PDA liposomes. This means PDA absorb NIR light and further it generates enough heat for anticancer effect. The high photothermal effects were shown by PDA (Fig. 4). The highest temperature of PDA solution reached to 69 ^{o}C after five minutes irradiation

at 808nm.

As previously mentioned above, PDA is a mussel mimicking adhesive proteins. Adhesiveness is relative to the cellular uptake efficiency [20,21] which means the existence of PDA can be influenced [22]. The cell internalization mechanisms of PDA have not been clarified [23], however, its cellular uptake was confirmed by confocal microscopy images (Fig. 5B, 5C). More uptake means higher photothermal anticancer effects due to the light energy PDA absorb itself and convert them into heat [1]. The temperature between 50 ^{o}C to 52 ^{o}C for four to six minutes may damage the cells by denaturing proteins [24]. At least, the temperature above 45 ^{o}C may kill tumor cells directly [1]. Therefore, the higher temperature and longer irradiation time may bring dramatic anticancer effects. However, if the temperature is too high for long time may bring serious burn in skin; so, finding the optimized irradiation time is unavoidable.

Not only PDA solution itself, also the cells treated with PDA-liposomes were displayed high photothermal activity (Fig.6). The results that the modified liposomes treated group was only responsive group to NIR light with regard to cellular uptake. The amount of the PDA adhesive to or internalized into the cell can be influenced its photothermal activities due to absorption of light energy [25].

As a result, treatment with the anticancer drug Edelfosine, encapsulated in liposomes, reduced viability of A375 human melanoma carcinoma cells compared to the cells treated with liposomes with no drug [26-29]. Also, the cellular damage have occurred with the PDA's photothermal activities. However, the anticancer effects of neither chemotherapy nor photothermal got

to the insufficient anticancer acitivity. Only the group with liposomes that carried of both Edelfosine and PDA showed lowest cell viability among other groups due to combinatorial therapies. Thus, PDA will be used and applied as a biocompatible material to the numerous way of developing multifunctional nano platforms in future medical field.

Ⅴ. Conclusion

In this study, we formulated liposomes, which were modified with PDA - mussel-inspired photoresponsive materials - for adherence and absorbance of NIR light to convert heat. These altered liposomes were then used as carriers for Edelfosine and NBD-PE, a fluorescent dye. The small size (nano-scale) of the modified liposomes enabled the enhanced permeability and retention effect (EPR) for targeting during *in vitro* studies. The results of this study suggested the usefulness of PDA-modification in the field of photothermal cancer therapy. Moreover, the technique utilizes combinatorial photothermal and chemotherapy, which can bring effective biomedical applications of multifunctional anticancer effect nanoparticles of high performance.

Ⅵ. References

1. Zhang Z, Wang J, Chen C. Near-infrared light-mediated nanoplatforms for cancer thermo-chemotherapy and optical imaging. Adv Mater. 2013;25:3869-80.
2. Lee SE, Liu GL, Kim F, Lee LP. Remote optical switch for localized and selective control of gene interference. Nano Lett 2009;9:562-70.Zhang Z, Wang J, Chen C. Near-infrared light-mediated nanoplatforms for cancer thermo-chemotherapy and optical imaging. Adv Mater. 2013;25:3869-80.
3. Maltzahn GV, Park JH, Agrawal A, Bandaru NK, Das SK, Sailor MJ, Bhatia SN. Computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas. Cancer Res. 2009;69:3892-900.
4. Iancu C, Mocan L. Advances in cancer therapy through the use of carbon nanotube-mediated targeted hyperthermia. Int J Nanomed. 2011;6:1675-84.
5. Choi WI, Kim JY, Kang C, Byeon CC, Kim YH, Tae G. Tumor regression in vivo by photothermal therapy based on gold-nanorod-loaded, functional nanocarriers. ACS Nano. 2011;5: 1995-2003.

6. Min Y, Mao C, Chen S, Ma G, Wang J, Liu Y. Combating the drug resistance of cisplatin using a platinum prodrug based delivery system. Angew Chem Int Ed. 2012;51:6742-47.

7. Helmchen F, Denk W. Deep tissue two-photon microscopy. Nat Methods. 2005;2:932-40.

8. Welsher K, Liu Z, Sherlock SP, Robinson JT, Chen Z, Daranciang D. A route to brightly fluorescent carbon nanotubes for near-infrared imaging in mice. Nat Nanotechnol 2009;4:773-80.

9. Wang Y, Black KC, Luehmann H, Li W, Zhang Y, Cai X. Comparison study of gold nanohexapods, nanorods, nanorods, and nanocages for photothermal cancer treatment. ACS Nano. 2013;7:2068-77.

10. Lin L, Cong Z, Cao J, Ke K, Peng Q, Gao J, Yang H, Liu G, Chen X. Multifunctional Fe_3O_4@Polydopamine Core-Shell Nanocomposites for Intracellular mRNA Detection and Imaging-Guided Photothermal Therapy. ACS Nano.

11. Zhang W, Guo Z, Huang D, Liu Z, Guo X, Zhong H. Synergstic effect of chemo-photothermal therapy using PEGylated graphene oxide. Biomaterials. 2012;336(6085):1124-1128.

12. Lee HS, Dellatore SM, Miller WM, Messersmith PB. Mussel-Inspired surface chemistry for multifunctional coatings. Science. 2007;318:426-30.

13. Sa R, Yan Y, Wei Z, Zhang L, Wang W, Tian M. Surface Modification of Aramid Fibers by Bio-inspired Poly(dopamine) and Epoxy Functionalized Silane Grafting. Appl Mater Interfaces. 2014;6:21730-38.

14. Lynge ME, Ogaki R, Laursen AO, Lovmand J, Sutherland DS, Stadler B. Polydopamine/Liposome coatings and their interation with myoblast cells. ACS Apppl Mater Interfaces. 2011;3:2142-47.

15. Liu Q, Wang N, Caro J, Huang A. Bio-inspired polydopamine: a versatile and powerful platform for covalent synthesis of molecular sieve membranes. J Am Chem Soc. 2013;135:17679-82.

16. Kim JH, Lee M, Park CB. Polydopamine as a Biomimetic Electron Gate for Artificial Photosynthesis. Angew Chem Int Ed. 2014;53:6364-6368.

17. Hong S, Kim KY, Hwang JW, Park SY, Lee KD, Lee DY, Lee HS. Attenuation of the *in vivo* toxicity of biomaterials by polydopamine surface modification. Nanomedicine. 2011;6:793-801.

18. Sa R, Yan Y, Wei Z, Zhang L, Wang W, Tian M. Surface Modification of Aramid Fibers by Bio-inspired Poly(dopamine) and Epoxy Functionalized Silane Grafting. Appl Mater Interfaces. 2014;6:21730-38.

19. Zhou J, Wang P, Wang C, Goh YT, Fang Z, Merssersmith PB, Duan H. Versatile core - Shell Nanoparitle@Metal - organic framework nanohybrids: exploiting mussle-inspired polydopamine for tailored structural integration. ACS Nano. 2015;9:6951-60.

20. Lesniak A, Salvati A, Santos-Martinez MJ, Radomski MW, Dawson KA, Aberg C. Nanoparticle adhesion to the cell membrane and its effect on nanoparticle uptake efficiency. J Am Cem Soc. 2013;135:1438-44.

21. Yuan H, Li J, Bao G, Zhang S. Variable nanoparticle-cell adhesion strength regulates cellular uptake. Phys Rev Lett. 2010;105:138101.

22. Lynge ME, Fernandez-Medina M, Postma A, Stadler B. Liposomal drug deposits in poly(dopamine) coatings: effect of their composition, cell type, uptake pathway considerations, and shear stress. Macromol Biosci. 2014;14:1677-87.

23. Lynge ME, Fernandez-Medina M, Postma A, Stadler B. Liposomal drug deposits in poly(dopamine) coatings: effect of their composition, cell type, uptake pathway considerations, and shear stress. Macromol Biosci. 2014;14:1677-87.

24. Zhou J, Chen JK, Zhang Y. Theoretical analysis of thermal damage in biological tissues caused by laser irradiation. Mol Cell Biomech. 2007;4:27-39.

25. Liu Y, Ai K, Liu J, Deng M, He Y, Lu L. Dopamine-Melanin

Colloidal Nanospheres: An efficient Near-Infrared photothermal therapeutic agent for *in vivo* cancer therapy. Adv Mater. 2013;52:2012-16.

26. Lee S, Kim J, Shim G, Kim S, Han SE, Kim K, Kwon IC, Choi Y, Kim YB, Kim CW, Oh YK. Tetraiodothyroacetic acid-tagged liposomes for enhanced delivery of anticancer drug to tumor tissue via integrin receptor. J Control Release. 2012;164:213-20.

27. Ahmand I, Filep JJ, Franklin JC, Janoff AS, Masters GR, Pattassery J, Peters A, Schupsky JJ, Zha Y, Mayhew E. Enhanced therapeutic effects of liposome-associated 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocoline. Cancer Res. 1997;57:1915-21.

28. Castro BM, Fedorov A, Hornillos V, Delgado J, Acuna AU, Mollinedo F, Prieto M. Edelfosine and miltefosine effects on lipid raft properties: membrane biophysics in cell death by antitumor lipids. J Phys Chem B 2013;117:7929-40.

29. Busto JV, Canto-Janez E, Goni FM, Mollinedo F, Alonso A. Combination of the anti-tumour cell ether lipid edelfosine with sterols abolishes haemolytic side effects of the drug. J Chem Biol. 2008;1:89-94.

Abstract (in Korean)

광감응 리포좀을 이용한 광열 항암 치료

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이 논문은 지질 기반 나노 전달체인 리포좀(liposome)을 광 감응성 물질로 코팅하여 암세포로의 전달을 통해 광열항암치료효과에 대해 연구를 수행하였다. 본 연구에서는 최근 큰 각광을 받고 있는 생체 모방 표면 개질 소재중 하나인 폴리도파민을 리포좀에 코팅하는 동시에 808nm 근적외선에 감응하는 물질로써 암세포 광열치료에 사용하였다. 도파민은 카테콜과 아민 작용기를 가지는 분자량 153(Da)의 단 분자 물질인데, 염기성 수용액상 조건 (pH 8.5)에서는 카테콜의 산화에 의해 자발적으로 반응이 진행되어 폴리도파민(polydopamine, pDA)을 형성하여 표면의 화학적 성질에 관계없이 금속, 고분자 등 다양한 소재 표면의 강하게 흡착되는 뛰어난 표면 부착능력이 있다. 홍합의 접착 메커니즘의 화학적 작용기만을 선택적으로 모방하여 도입한 생체 모방 표면 개질 기법 (bio-inspired

surface modification)은 접착성뿐만 아니라 생체재료의 가장 중요한 요소인 생체친화적인 (biocompatible) 코팅 기법으로써 이미 다양한 선행연구들에서 표면개질효능과 세포독성이 없다는 점이 세포배양실험을 통해 밝혀져 왔다. 본 연구는 선행연구와의 차별성과 학술적 발전에 기여하기 위하여 리포좀 표면의 폴리도파민을 흡착시켜 코팅 했을 뿐만 아니라 암세포로 전달한 후, 외부에서 근적외선을 조사하여 광열효과를 유도하였다. 대조군과 비교했을 때 광감응성 리포좀의 온도는 최고 69 ℃까지 올라 세포실험 결과 암세포들이 사멸하는 것을 확인하였다. 또한 현재 널리 쓰이고 있는 항암제인 Edelfosine을 리포좀에 봉입하여 폴리도파민으로 코팅하고 암세포에 전달한 뒤 근적외선의 광열효과를 통해 세포생존율의 감소를 확이 할 수 있었다. 본 연구의 의의는 리포좀을 이용하여 항암제를 전달하고 동시에 광 감응성 물질을 코팅함으로써 근적외선 조사에 의한 광열효과까지 갖춘 리포좀을 개발하였다. 이러한 광감응성 리포좀은 봉입한 항암제에 의한 화학요법과 함께 광열치료의 상승작용을 기대할 수 있어 미래의 새로운 항암 의약재료의 가능성을 확인하였다.

Key words: Liposomes, Near infrared light, Bio-inspired surface modification, Biocompatibility, Photothermal therapy, Chemotherapy

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