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ARTICLE TYPE

Multifunctional Polydiacetylene-Liposome with Controlled Release and Fluorescence Tracing

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A multifunctional polydiacetylene-liposome (PDA-liposome) was prepared by self-assembly, which can be characterized with controlled drug release and fluorescence tracing. The drug control release was realized both in vitro simulation and cancer cells by temperature control. The internalization and distribution of the PDA-liposome in cancer cells were presented by fluorescence cell imaging.

Theranostic was defined as a material that combines the modalities of therapy and diagnostic imaging by Funkhouser in 2002.[1] So far, it has been developed a lot and facilitated advances in medical research.[2] In contrast to the development and use of separate materials for disease therapy and diagnosis, theranostics combine features of delivering therapeutic drugs and diagnostic imaging into one entity, which has the potential to overcome the undesirable drug release and biological toxicity in current distinct imaging and therapeutic agents.[3] The ultimate goal for the theranostic field is to gain the ability to image and monitor the diseased tissue, delivery kinetics, and improve the drug efficacy by controlled release.[4] The development of nanomaterials,[5] including liposomes, polymers, micells, inorganic nanoparticles, and peptide/protein conjugates, offers new opportunities to meet the need of theranostics.[6] The most promising aspects of utilizing nanoparticles as therapeutics, diagnostics, and theranostics are their potential to localize (or be targeted) in a specific manner to the site of disease and reduce or eliminate the possible numerous untoward side effects.[7, 8] The functional nanoparticles have been investigated in controlled release,[9] site-specific targeting,[10, 11] or imaging[12-14] for potential applications in cancer therapy and diagnosis. However, there are several inherent disadvantages with current formulations in drug delivery and imaging, including toxicity to living organisms,[15, 16] fluorescence bleaching, severe side effects, uncontrolled drug release, instability in storage, and so on. A biocompatible drug carrier with integrated features of controlled release and imaging is urgently needed for biological and medical research.

Liposomes are spherical vesicles consisting of phospholipid bilayers surrounding an aqueous cavity.[17] The biocompatible liposomes in nanoscale have been used widely for drug delivery,[18] and there are several liposome-mediated drug delivery products approved for clinical trials.[19] Many current methods by incorporating components are applied to achieve controlled drug release of liposomes with thermal,[20] photo-thermal,[21] pH,[22] magnetic,[23] or enzymatic triggers.[24] Thermosensitive liposomes with a lower critical solution temperature (T_c) are common drug carriers that function by thermo-stimulated drug release, and it has been used in various cancer treatments.[25] The T_c is the phase transition temperature for gel to liquid crystalline transition in the liposomes.[26] It has been found that nanocarrier mediated delivery of anticancer drugs can be positively influenced by localized thermal treatment.[27]

Polydiacetylene (PDA), a class of eneyne conjugated polymers derived from amphiphilic monomers of diacetylenic acid, has been extensively investigated on its peculiar optical properties.[28, 29] Recently, more attention was paid to the fluorescence property of PDA in sensing and detections.[30] However, the intrinsic poor hydrophilicity of PDA restricted the applications in aqueous solutions. PDA liposome composed of PDA and phospholipids possesses a good solubility and stability and has been used in drug delivery as a drug sustained-release system.[31, 32] To the best of our knowledge, a combination of thermo-triggered drug release and fluorescence tracing in PDA liposome has not been reported yet.

The goal of this work is to present a drug delivery system of polydiacetylene liposome (PDA liposome) with multifunctional properties of thermo-triggered release and fluorescence tracking. To the best of our knowledge, no studies have been reported on the drug controlled release at the cellular level yet. Docetaxel (DTX), a semisynthetic derivative of the taxoid family of antineoplastic agents,[33] was used as a model drug. The morphology, size, entrapment efficiency, and fluorescence property of DTX-loaded PDA liposomes (DLPLs) were discussed. The thermo-triggered release of DLPLs was studied *in vitro* simulating experiments and model cancer cells. The internalization and distribution of the DLPLs in cancer cells were monitored by fluorescence cell imaging.

DLPLs were prepared by self-assembly using a combination method of thin-film hydration and supercritical CO_2 fluid (SFC-CO_2),[34] as shown in electronic supplementary information (ESI). A series of PDA liposomes with different PDA/Lipid ratios were prepared as shown in ESI, and the optimal mole ratio of PDA/phospholipids was determined as 0.8. So sample with ratio of 0.8 was used for various studies in the manuscript. The morphology of DLPLs was characterized by transmission electron microscope (TEM), as shown in Fig. 1a. The TEM image revealed that the DLPLs were monodisperse micro-spheres, and the microstructure was shown more clearly in the inset. The size distribution of DLPLs was determined by dynamic light

scattering (DLS) at 25°C. As shown in Fig. 1b, the average size of DLPLs was about 250 ± 15 nm. DLPLs with this size were suitable for drug delivery in cancer therapy by the enhanced permeability and retention (EPR) effect.[35, 36]

The entrapment efficiency of DLPLs was determined by high performance liquid chromatography (HPLC) using dialysis method as shown in electronic supplementary information (ESI). DTX entrapment efficiency in DLPLs was determined[21], and which was about 96.5 ± 1.0 %.

In order to explore the effect of temperature on drug release of DLPLs, *in vitro* release studies were carried out at various temperatures. The cumulative release profiles of DLPLs at different temperatures were shown in Fig. 2(a). The drug release was found to be greatly dependent on temperature. As shown in Fig. 2(b), the drug release increased with increasing temperatures at temperature below 40 °C, and which decreased with increasing temperatures at temperature above 40 °C at release time of 300 min. The drug was released abundantly from DLPLs at an optimal temperature of 40°C. To explain the temperature-dependent release mechanism, lower critical solution temperature (T_c) of DLPLs was measured by micro-differential scanning calorimetry (micro-DSC). As shown in Fig. 2(c), the T_c of DLPLs was 40 ± 1°C, which was consistent with the optimum drug release temperature of DLPLs within the experimental error. The encapsulated drug was released abruptly from the PDA liposome at T_c because of the enhanced permeability of lipid bilayer membrane, which was resulted from the gel-liquid crystalline phase transition of DLPLs. Whereas when temperature was below or above T_c, the drug release would be hindered by tight arrangement or blocked by partially parallel arrangement of phospholipids in the membranes of DLPLs, respectively.[22] The thermo-triggered release drug can be realized through combination process of light irradiation and photothermal conversion.[21] The results will provide new therapy of hyperthermia and photo-thermal therapy for cancer care.

The fluorescence property of PDA liposome is an essential parameter in cell imaging and tracking. The fluorescence emission spectra of PDA liposome and PDA vesicle solutions were measured by fluorescence spectroscopy at 25°C. As shown in Fig. 3a, the fluorescence intensity of PDA liposome in aqueous solution was much higher than that of PDA vesicle because the solubility of PDA was improved a lot by introducing phospholipids in the PDA liposome.

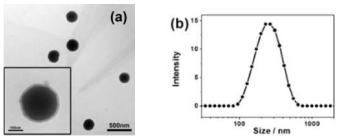

Fig. 1 (a) TEM images and (b) size distribution of DLPLs.

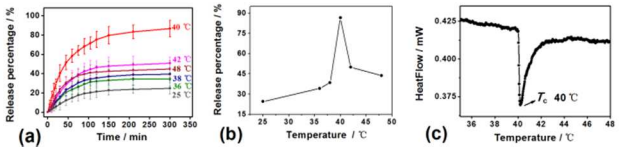

Fig. 2 (a) Cumulative release profiles of DLPLs at different temperatures; (b) The plot of cumulative drug release vs. temperature at release time of 300 min; (c) Heating curve of DLPLs measured by micro-DSC at scanning rate of 1 °C/min.

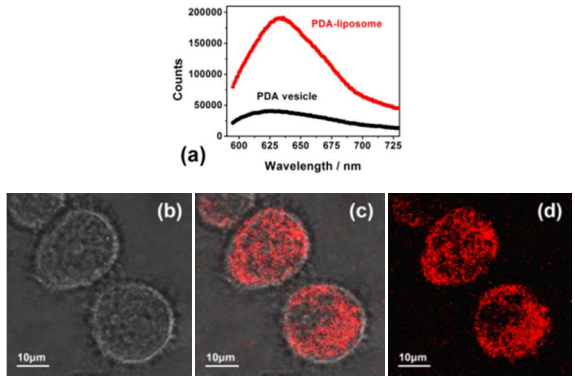

Fig. 3 (a) Fluorescence emission spectra of PDA liposome (red line) and PDA vesicle (black line) in aqueous solution at 298K; (b-d) LCSM images of Bcap-37 cells treated with DLPLs for 2 h: (b) bright-field image; (c) composite bight-field and fluorescence image; (d) fluorescence image. All images were obtained with a 63× objective.

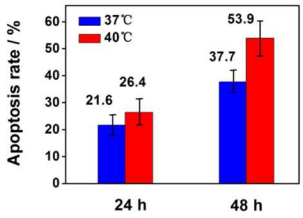

Fig. 4 Apoptosis rates of Bcap-37 cells incubated with DLPLs by common release at 37°C or thermo-triggered release at 40°C after 24 h and 48 h, respectively. (Blue columns: 37°C and red columns: 40°C)

In order to investigate the biocompatibility and drug efficacy of DLPLs, human breast carcinoma cells (Bcap-37) were selected as model cells for live cell imaging and cytotoxicity tests. The fluorescence cell imaging was performed using laser scanning confocal microscopy (LSCM). Trans-membrane behaviour and distribution of DLPLs were monitored by fluorescence cell imaging. After incubation for 2 h at 37°C in humidified atmosphere containing 5.0 % CO_2, the DLPLs had been internalized inside the Bcap-37 cells, as shown in Fig. 3b-d. It revealed that DLPLs were provided with good membrane permeability. By the transmembrane and endocytic behaviours, the drug was concentrated effectively in cancer cells, and the anticancer efficacy of DTX could be improved markedly.

Before evaluating the anticancer efficacy of DLPLs, cytotoxicity of blank PDA liposome was tested on Bcap-37 cells by MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays and flow cytometry (FCM), as shown in ESI. It was indicated that blank PDA liposome had very little cytotoxicity to Bcap-37 cells.

The DTX in DLPLs was released in Bcap-37 cells by temperature control to evaluate the effect of temperature on the controlled release of DLPLs. Bcap-37 cells were incubated with DLPLs for 2 h before the controlled release treatment to ensure the DLPLs transmembrane transportation. The Bcap-37 cells were separated into two groups. One experimental group (G1) was treated at 40°C for 30 min, the other control group (G2) was treated at the common culture temperature (37°C), followed by incubation at 37°C for 24 h or 48 h, respectively. After the incubation, the cell apoptosis rates were measured by FCM. As shown in Fig. 4, the apoptosis rates of Bcap-37 cells in the G1

were 21.6 ± 3.9% and 26.4 ± 4.5% at 24 h and 48 h, respectively. However, the apoptosis rates were increased to 37.7 ± 6.1% and 53.9 ± 6.4% in the G2 at 24 h and 48 h, respectively. Comparing to the control group (G1), the apoptosis rates of Bcap-37 cells in the G2 were increased by 4.8% and 16.2% for 24 h and 48 h, respectively. It means that the drug efficacy of DLPLs using thermal stimulus controlled release was about 1.2 and 1.4 times of that using common release for 24 h and 48 h, respectively. The results of thermal stimulus controlled drug release in the DLPLs prove that the drug efficacy at T_c temperature of DLPLs is higher than that at 37 °C, and which is in accordance with that in vitro model experiments (Fig 2). This phenomenon could be caused by synergistic effects of thermo-triggered release of DLPLs and good permeability of membrane. The fluorescence thermal stimulus PDA liposome will be a potential controlled release system for drug delivery and tracking.

Conclusions

In summary, this work presented a novel DLPLs drug delivery system, which provides both function of controlled drug release and a fluorescence tracking. The DLPLs were characterized with good biocompatibility, membrane permeability, high drug entrapment efficiency, enhanced fluorescence, and thermo-triggered drug release. The temperature-controlled drug release has been realized both in vitro simulation and cancer cells. Significantly, the anticancer drug efficacy was much improved using temperature-controlled drug release in Bcap-37 breast cancer cells. The DLPLs can be accumulated in Bcap-37 breast cancer cells within 2 h. The intracellular distribution of DLPLs could be traced and monitored by fluorescence imaging in real time. This technology could be realized in oncotherapy of surface tumors. The results showed that there is still great space for developing, and it will be further studied to obtain a novel targeted delivery system in vivo, which is integration system of both treatment and diagnosis. The novel DLPLs could be a promising candidate for drug delivery, disease diagnosis and therapy.

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Notes and references

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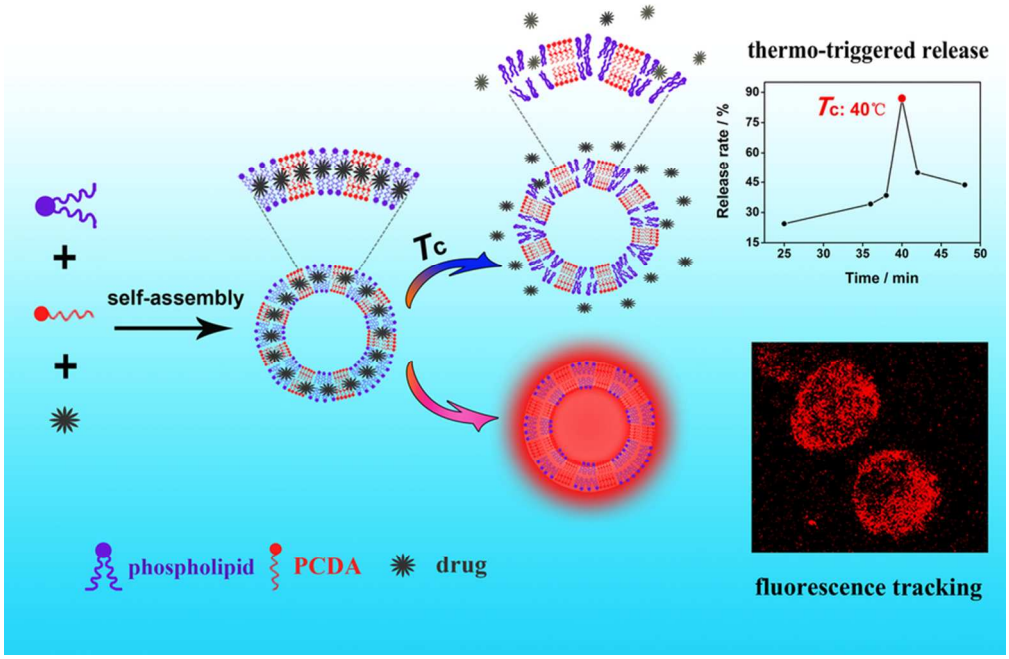
Electronic Supplementary Information (ESI) available: [Preparation methods of PDA liposome, dialysis method, fluorescence cell imaging, phase transition temperatures, cell culture, and cytotoxicity of PDA liposome]. See DOI: 10.1039/c000000x/

1. J. Funkhouser, *Curr. Drug Discovery* 2002, **2**.
2. T. Lammers, S. Aime, W. E. Hennink, G. Storm and F. Kiessling, *Acc. Chem. Res.*, 2011, **40**, 1029-1038.
3. M. E. Caldorera-Moore, W. B. Liechty and N. A. Peppas, *Acc. Chem. Res.*, 2011, **44**, 1061-1070.
4. S. S. Kelkar and T. M. Reineke, *Bioconjugate Chem.*, 2011, **22**, 1879-1903.
5. V. P. Torchilin, *Adv. Drug Delivery Rev.*, 2006, **58**, 1532-1555.
6. T. L. Doane and C. Burda, *Chem. Soc. Rev.*, 2012, **41**, 2885-2911.
7. H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi and J. V. Frangioni, *Nat. Biotechnol.*, 2007, **25**, 1165-1170.
8. S. Santra, C. Kaittanis and J. Grimm, *Small* 2009, **5**, 1862-1868.
9. L. Paasonen, T. Laaksonen, C. Johans, M. Yliperttula, K. Kontturi and A. Urtti, *J. Control Release*, 2007, **122**, 86-93.
10. L. D. Leserman, J. Barbet, F. Kourilsky and J. N. Weinstein, *Nature*, 1980, **288**, 602-604.
11. N. Mackiewicz, E. Gravel, A. Garofalakis, J. Ogier, J. John, D. M. Dupont, K. Gombert, B. Tavitian, E. Doris and F. Ducongé, *Small*, 2011, **7**, 2786-2792.
12. O. Veiseh, C. Sun, J. Gunn, N. Kohler, P. Gabikian, D. Lee, N. Bhattarai, R. Ellenbogen, R. Sze, A. Hallahan, J. Olson and M. Zhang, *Nano Lett.*, 2005, **5**, 1003-1008.
13. L. Cheng, K. Yang, Y. Li, J. Chen, C. Wang, M. Shao, S.-T. Lee and Z. Liu, *Angew. Chem.*, 2011, **123**, 7523 -7528.
14. D. Kim, Y. Y. Jeong and S. Jon, *ACS Nano*, 2010, **4**, 3689-3696.
15. A. M. Derfus, W. C. W. Chan and S. N. Bhatia, *Nano Lett.*, 2004, **4**, 11-18.
16. R. Hardman, *Environ. Health Perspect.*, 2006, **114**, 165-172.
17. K. Cho, XuWang, S. Nie, Z. G. Chen and D. M. Shin, *Clin. Cancer Res.*, 2008, **14**, 1310-1316.
18. W. T. Al-Jamal and K. Kostarelos, *Acc. Chem. Res.*, 2011, **44**, 1094-1104.
19. V. P. Torchilin, *Nat. Rev. Drug Discov.*, 2005, **4**, 145-160.
20. A. Agarwal, M. A. Mackey, M. A. El-Sayed and R. V. Bellamkonda, *ACS Nano*, 2011, **5**, 4919-4926.
21. X. An, F. Zhang, and Y. Zhu, *Langmuir*, 2013, **29**, 1061-1068.
22. W. Zhou, X. An, J. Wang, W. Shen, Z. Chen and X. Wang, *Colloids Surf., A*, 2012, **395**, 225- 232.
23. D. Qiu and X. An, *Colloids Surf., B*, 2013, **104**, 326-329.
24. J. Davidsen, K. Jorgensen, T. L. Andresen and O. G. Mouritsen, *Biochim. Biophys. Acta, Lipids Lipid Metab.*, 2003, **1609**, 95-101.
25. D. Needham and M. W. Dewhirst, *Adv. Drug Delivery Rev.*, 2001, **53**, 285-305.
26. D. Chapmana, *Q. Rev. Biophys.*, 1975, **8**, 185-235.
27. S. Ganta, H. Devalapally, A. Shahiwala and M. Amiji, *J. Control Release*, 2008, **126**, 187-204.
28. D. H. Charych, J. O. Nagy, W. Spevak and M. D. Bednarski, *Science*, 1993, **261**, 585-588.
29. S. Wacharasindhu, S. Montha, J. Boonyiseng, A. Potisatityuenyong, C. Phollookin, GamolwanTumcharern and M. Sukwattanasinitt, *Macromolecules*, 2010, **43**, 716-724.
30. J. Wu, A. Zawistowski, M. Ehrmann, T. Yi and C. Schmuck, *J. Am. Chem. Soc.*, 2011, **133**, 9720-9723.
31. C. Guo, S. Liu, Z. Dai, C. Jiang and W. Li, *Colloids Surf., B*, 2010, **76**, 362-365.
32. C. Guo, S. Liu, C. Jiang, W. Li, Z. Dai, H. Fritz and X. Wu, *Langmuir*, 2009, **25**, 13114-13119.
33. M.-C. Bissery, D. Guenard, F. Gueritte-Voegelein and F. Lavelle, *Cancer Res.*, 1991, **51**, 4845-4852.
34. X. An, F. Zhang, Y. Zhu and W. Shen, *Chem. Commun.*, 2010, **46**, 7202-7204.

35. A. K. Iyer, G. Khaled, J. Fang and H. Maeda, *Drug Discovery Today*, 2006, **11**, 812-818.
36. H. Maeda, K. Greish and J. Fang, *Adv. Polym. Sci.*, 2006, **193**, 103-121.



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