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Layered double hydroxide bio-composites toward excellent systematic anticancer therapy†

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Multi-therapeutic methodologies have attracted considerable attention toward cancer therapy, which can overcome the limitation of a single therapy and achieve an optimized anticancer efficacy. Herein, we prepared a systematic anticancer drug by intercalating zinc phthalocyanines (ZnPc) into a layered double hydroxide (LDH) gallery, followed by loading doxorubicin (DOX) on the surface (denoted as ZnPc-DOX/LDH). ZnPc is accommodated in the interlayer region of the LDH, which results in a largely enhanced photodynamic therapeutic (PDT) efficiency; while the physisorbed DOX affords a chemotherapeutic effect. In vitro tests performed with KB cells indicate a synergistic anticancer performance as well as excellent biocompatibility compared with pristine ZnPc and DOX. This study demonstrates a promising bio-composite for PDT–chemotherapy systematic therapy, which shows potential application in the field of cancer therapy.

Introduction

Recently, therapeutic methodologies toward cancer have been widely explored[1,2] owing to the high mortality rate in the world. The current therapeutic methods include surgery, chemotherapy, radiotherapy, hyperthermia and photodynamic therapy (PDT).[3–7] However, each individual therapeutic approach always shows certain intrinsic limitations.[8,9] For instance, as a highly-efficient treatment, chemotherapy displays a dominant modality among all the cancer therapies, but suffers from serious side effects to normal tissues and organs due to their powerful and non-selective activities.[10–13] Another example is photodynamic therapy (PDT), which has appeared as a potential approach for its non-invasiveness and high selectivity compared with traditional cancer treatment methods.[14,15] In PDT, a photosensitizer (PS) can be elevated to the excited state by light irradiation, and then interacts with oxygen to generate singlet oxygen ($^1O_2$) or other reactive oxygen species (ROSs), which consequently causes oxidative damage to tumor cells.[16] However, the treatment depth remains an obstacle for the further development of PDT.[17] Taking all these factors into consideration, it is a highly desirable strategy to combine PDT and chemotherapy with a drug delivery vehicle, so as to overcome the limitation of a single therapy and achieve an optimized anticancer efficacy with depressed side effects.[18–20]

Layered double hydroxides (LDHs), a class of naturally occurring and synthetic materials generally expressed by the formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2](A^{n-})_{x/n}\cdot mH_2O$, have attracted increasing attention due to their unique 2D structure with tenability in both the host layer and interlayer anions.[21–24] As reported, LDHs are good nanocarriers for drug delivery with excellent biocompatibility and extremely low cytotoxicity.[25,26] The host layer of LDHs will dissolve when the pH value is less than 5.5, which makes LDHs superior nanocarriers for pH controlled drug release.[27] In addition, LDHs can effectively enhance the cellular uptake capacity via drug intercalation due to the electropositivity of the host layer, which results in an improved passive targeting ability.[28–30]

Herein, we report bio-composite ZnPc-DOX/LDH nanoparticles by the intercalation of zinc phthalocyanines (ZnPc) into an LDH gallery, followed by the loading of doxorubicin (DOX) to achieve a synergistic chemotherapy–PDT cancer therapy. XRD patterns, FT-IR and UV-vis spectra confirm the successful intercalation of ZnPc into the LDH gallery with a monomer state, which is a key factor for its singlet oxygen production efficiency. The release behaviour indicates that ZnPc-DOX/LDH possesses pH controlled release performance. In vitro tests performed with KB cells exhibit an excellent chemotherapy–PDT synergistic therapeutic performance compared with the individual cancer therapeutics. In addition, the biocompatibility of ZnPc-DOX/LDH is demonstrated, which serves as a promising candidate in the systematic anticancer therapy field.

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† Electronic supplementary information (ESI) available: FT-IR spectra are displayed in Fig. S1. The SEM images and DLS results are shown in Fig. S2 and S3. The zeta potential distribution is presented in Fig. S4. The UV-vis absorption spectra are shown in Fig. S5–S8. The fluorescence intensity and fluorescence image for KB cells are displayed in Fig. S9. Fig. S10 shows the KB cells stained with PI. Fig. S11 displays KB cells incubated with ZnPc-DOX/LDH under partial irradiation. See DOI: 10.1039/c7tb00209b

Results and discussion

ZnPc-DOX/LDH nanoparticles were synthesized by the separate nucleation and aging steps (SNAS) method,[31] via the intercalation of ZnPc (tetrasulfonated phthalocyanine) into a gallery of LDHs. Subsequently, DOX was further adsorbed on the exterior surface through electrostatic interaction. ZnPc/LDH and DOX/LDH as reference samples, were also prepared by the intercalation of ZnPc and electrostatic absorption of DOX, respectively. Scheme 1 illustrates the fabrication process and structure of ZnPc-DOX/LDH. In our previous work, the best intercalated ratio of ZnPc was determined to be 1.5%;[32] thus, the ZnPc/LDH and ZnPc-DOX/LDH were prepared with 1.5% ZnPc content (it is defined as the molar ratio of ZnPc : Al). The XRD patterns of the DOX/LDH, ZnPc/LDH and ZnPc-DOX/LDH samples are shown in Fig. 1A. For the sample of DOX/

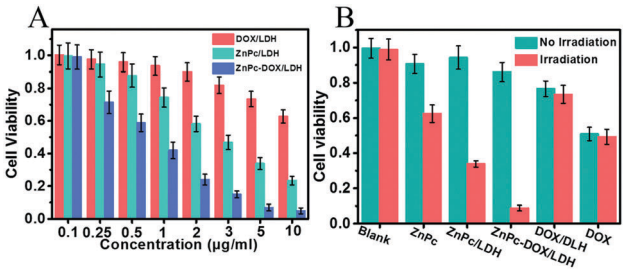

Fig. 5 Anticancer performance of (A) DOX/LDH, ZnPc/LDH and ZnPc-DOX/LDH with various concentrations after 24 h of incubation, and (B) anticancer performance of these composites with equivalent total drugs (5 μg mL^{-1}) after 24 h of incubation.

The anticancer performance of ZnPc/LDH, DOX/LDH and ZnPc-DOX/LDH was tested in cellular experiments by using KB cells. In a concentration-dependent manner, KB cells were seeded in a 96-well plate, followed by incubation with ZnPc/LDH, DOX/LDH and ZnPc-DOX/LDH at a series of concentrations of total drugs for 24 h. Subsequently, the wells were irradiated with a 650 nm NIR light at a power density of 10 mW cm^{-2} for 20 min. The relative viabilities of the cells were determined using a standard methyl thiazolyl tetrazolium (MTT) assay. Fig. 5A shows that increasing cell death occurs as the total drug concentration increases from 0.1 to 10 μg mL^{-1}, indicating the significant anticancer performance. The half maximal inhibitory concentration (IC_{50}) was calculated to be 18.2, 2.89 and 0.605 μg mL^{-1} for DOX/LDH, ZnPc/LDH and ZnPc-DOX/LDH, respectively. The anticancer efficiency of ZnPc-DOX/LDH is obviously superior to that of DOX/LDH and ZnPc/LDH with the same total drug concentration, indicating the excellent chemotherapy-PDT synergistic therapeutic performance. The synergistic effect will be discussed in the following section. Moreover, to further understand such superior anticancer performance, the cellular uptake behavior of ZnPc-DOX/LDH was studied. KB cells were incubated with ZnPc-DOX/LDH with drug concentrations from 0.5 to 5 μg mL^{-1} for 24 h, and their fluorescence images were recorded by a fluorescence microscope. As shown in Fig. S9A (ESI†), the fluorescence intensity of ZnPc-DOX/LDH in the cell lysate sample increases gradually along with the increment of drug concentration and the fluorescence image of the sample incubated with 5 μg mL^{-1} of ZnPc-DOX/LDH exhibits bright luminance (Fig. S9B, ESI†). This result indicates that the electropositivity and suitable nano-composite structure of ZnPc-DOX/LDH endow an enhanced uptake through passive targeted internalization.

For comparison, KB cells were incubated with blank sample, ZnPc, ZnPc/LDH, DOX, DOX/LDH and ZnPc-DOX/LDH with a concentration of 5 μg mL^{-1}, respectively. As shown in Fig. 5B, irradiation has no influence on the cells as the blank group revealed. For pristine ZnPc, the cell viability before and after irradiation is 0.907 and 0.625 respectively, indicating its slight cytotoxicity and weak PDT effectiveness. The cell viability of ZnPc/LDH changes from 0.943 to 0.338 upon irradiation, which demonstrates the greatly-enhanced PDT effectiveness and extremely low cytotoxicity. For pristine DOX, it exhibits an obvious cytotoxicity toward cells (cell viability: 0.509) because of the rapid cellular uptake. The cytotoxicity of DOX/LDH (cell viability: 0.767) is lower than DOX for the reason that the former sample would undergo an uptake-delivery-release procedure. The cell viability of ZnPc-DOX/LDH is 0.861 before irradiation, revealing its low cytotoxicity. After irradiation, the cell viability declines to 0.088, demonstrating its excellent anticancer efficiency in virtue of combining chemotherapy and PDT.

For the purpose of visualizing the cancer therapeutic results, KB cells were stained with propidium iodide (PI), which can only get through the membrane of dead cells and is excluded from viable cells. The cytotoxicity of ZnPc, ZnPc/LDH, ZnPc-DOX/LDH and the blank sample was firstly investigated without irradiation. Fig. S10A–C (ESI†) show that no obvious cell mortality is observed for the blank, pristine ZnPc and ZnPc/LDH, while ZnPc-DOX/LDH leads to cell mortality to some extent (Fig. S10D, ESI†). Fig. 6 displays fluorescence images of KB cells incubated with 5 μg mL^{-1} of ZnPc, ZnPc/LDH, ZnPc-DOX/LDH, DOX, DOX/LDH and blank with irradiation. The blank group reveals that the irradiation has no influence on the cells (Fig. 6A). Fig. 6B–C show the partial PI signal in the groups of DOX and DOX/LDH due to the chemotherapy of DOX. The PI signal of cells incubated with pristine ZnPc is extremely weak, indicating the weak PDT efficiency (Fig. 6D). Cells incubated with ZnPc/LDH (Fig. 6E) display a much stronger PI signal, illustrating an obviously enhanced PDT efficiency. As shown in Fig. 6F, the introduction of ZnPc-DOX/LDH leads to excellent anticancer performance because of the synergistic effect of combined chemotherapy-PDT, which is in good agreement with the in vitro tests above. The synergistic effect of combined chemotherapy-PDT was also demonstrated by a consecutive treatment. KB cells treated with chemotherapy undergo a weak anticancer performance but obviously decreased cell activity and distorted cytoderm (Fig. S11A, ESI†); a further implement of PDT results in the final death of the KB cells (Fig. S11B, ESI†), accounting for the largely improved anticancer therapy.

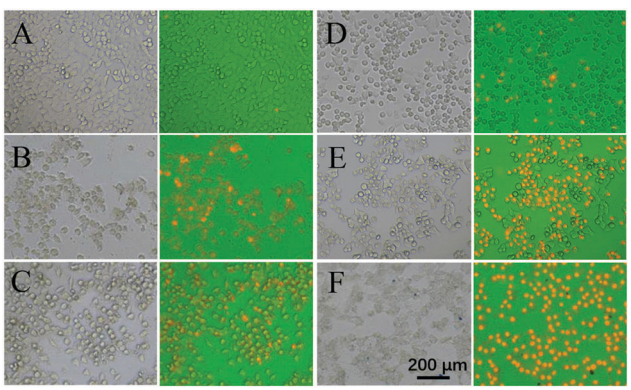

Fig. 6 Bright field (left column) and merged (right column) images of KB cells treated with various composites with irradiation (5 μg mL^{-1}, 24 h incubation): (A) blank; (B) DOX; (C) DOX/LDH; (D) ZnPc; (E) ZnPc/LDH; (F) ZnPc-DOX/LDH.

Conclusions

In summary, a supramolecular bio-composite toward systematic chemotherapy-PDT therapy was fabricated via incorporation of ZnPc within an LDH gallery, followed by further adsorption of DOX. The host…guest interactions of ZnPc and the LDH result in the monomeric state of ZnPc in the interlayer region of the LDH matrix, with a large singlet oxygen production efficiency. In vitro tests performed with KB cells reveal that the ZnPc-DOX/LDH composite exhibits a satisfactory anticancer effectiveness (equivalent 2.5 mg mL^{-1} ZnPc and 2.5 mg mL^{-1} DOX result in 91.2% cell death), good biocompatibility as well as low cytotoxicity, in comparison with pristine ZnPc and DOX. In addition, the excellent chemotherapy-PDT synergistic effect and superior passive targeted ability of ZnPc-DOX/LDH is the most distinct feature. Therefore, this work provides a facile approach for the design and preparation of a bicomponent material with largely-enhanced anticancer behavior, which can serve as a promising composite drug in the field of tumor therapy.

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Notes and references

1 L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-Tieulent and A. Jemal, Ca-Cancer J. Clin., 2015, 65, 87…108.
2 J. Brahmer, K. L. Reckamp, P. Baas, L. Crino, W. E. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E. E. Vokes, E. Holgado, D. Waterhouse, N. Ready, J. Gainor, O. Aren Frontera, L. Havel, M. Steins, M. C. Garassino, J. G. Aerts, M. Domine, L. Paz-Ares, M. Reck, C. Baudelet, C. T. Harbison, B. Lestini and D. R. Spigel, N. Engl. J. Med., 2015, 373, 123…135.
3 P. Sharma and J. Allison, Science, 2015, 348, 56…61.
4 B. J. Solomon, T. Mok, D. W. Kim, Y. L. Wu, K. Nakagawa, T. Mekhail, E. Felip, F. Cappuzzo, J. Paolini, T. Usari, S. Iyer, A. Reisman, K. D. Wilner, J. Tursi, F. Blackhall and P. Investigators, N. Engl. J. Med., 2014, 371, 2167…2177.
5 L. Deng, H. Liang, B. Burnette, M. Beckett, T. Darga, R. R. Weichselbaum and Y. X. Fu, J. Clin. Invest., 2014, 124, 687…695.
6 Y. Yuan, C. J. Zhang, M. Gao, R. Zhang, B. Z. Tang and B. Liu, Angew. Chem., Int. Ed., 2015, 54, 1780…1786.
7 R. Mejia-Ariza, L. Grana-Súarez, W. Verboom and J. Huskens, J. Mater. Chem. B, 2017, 5, 36…52.
8 W. Fan, P. Huang and X. Chen, Chem. Soc. Rev., 2016, 45, 6488…6519.
9 C. Bailly, Chem. Rev., 2012, 112, 3611…3640.
10 C. S. Cutler, H. M. Hennkens, N. Sisay, S. Huclier-Markai and S. S. Jurisson, Chem. Rev., 2013, 113, 858…883.
11 C. Liang, S. Diao, C. Wang, H. Gong, T. Liu, G. Hong, X. Shi, H. Dai and Z. Liu, Adv. Mater., 2014, 26, 5646…5652.
12 Y. Zhou, Y. Dong, G. Huang, Y. Wang, X. Huang, F. Zhang, D. A. Boothman, J. Gao and W. Liang, J. Mater. Chem. B, 2016, 4, 7429…7440.
13 P. Viens, C. Tarpin, H. Roche and F. Bertucci, Cancer, 2010, 116, 2829…2836.
14 J. Ge, M. Lan, B. Zhou, W. Liu, L. Guo, H. Wang, Q. Jia, G. Niu, X. Huang, H. Zhou, X. Meng, P. Wang, C. S. Lee, W. Zhang and X. Han, Nat. Commun., 2014, 5, 4596…4604.
15 L. Feng, F. He, G. Yang, S. Gai, Y. Dai, C. Li and P. Yang, J. Mater. Chem. B, 2016, 4, 8000…8008.
16 Z. Zhou, J. Song, L. Nie and X. Chen, Chem. Soc. Rev., 2016, 45, 6597…6626.
17 L. Zhao, H. Yang, T. Amano, H. Qin, L. Zheng, A. Takahashi, S. Zhao, I. Tooyama, T. Murakami and N. Komatsu, J. Mater. Chem. B, 2016, 4, 7741…7748.
18 Q. Chen, C. Wang, L. Cheng, W. He, Z. Cheng and Z. Liu, Biomaterials, 2014, 35, 2915…2923.
19 R. Liang, S. You, L. Ma, C. Li, R. Tian, M. Wei, D. Yan, M. Yin, W. Yang, D. G. Evans and X. Duan, Chem. Sci., 2015, 4, 5511…5518.
20 L. Guo, D. Yan, D. Yang, Y. Li, X. Wang, O. Zalewski, B. Yan and W. Lu, ACS Nano, 2014, 8, 5670…5681.
21 Y. Zhao, C. J. Wang, W. Gao, B. Li, Q. Wang, L. Zheng, M. Wei, D. G. Evans, X. Duan and D. O•Hare, J. Mater. Chem. B, 2013, 1, 5988…5994.
22 Q. Wang and D. O•Hare, Chem. Rev., 2012, 112, 4124…4155.
23 D. G. Evans and X. Duan, Chem. Commun., 2006, 485…496.
24 R. Liang, M. Wei, D. G. Evans and X. Duan, Chem. Commun., 2014, 50, 14071…14081.
25 S. Li, J. Li, C. J. Wang, Q. Wang, M. Z. Cader, J. Lu, D. G. Evans, X. Duan and D. O•Hare, J. Mater. Chem. B, 2013, 1, 61…68.
26 L. Li, Z. Gu, W. Gu, J. Liu and Z. P. Xu, J. Colloid Interface Sci., 2016, 470, 47…55.
27 S. Barkhordari, M. Yadollahi and H. Namazi, J. Polym. Res., 2014, 21, 454…463.
28 A. W. Musumeci, G. M. Mortimer, M. K. Butler, Z. P. Xu, R. F. Minchin and D. J. Martin, Appl. Clay Sci., 2010, 48, 271…279.
29 D. S. Tong, M. Liu, L. Li, C. X. Lin, W. H. Yu, Z. P. Xu and C. H. Zhou, Appl. Clay Sci., 2012, 70, 1…7.
30 C. Vasti, D. A. Bedoya, R. Rojas and C. E. Giacomelli, J. Mater. Chem. B, 2016, 4, 2008…2016.
31 L. Wang, X. Xu, D. G. Evans, X. Duan and D. Li, J. Solid State Chem., 2010, 183, 1114…1119.
32 R. Liang, R. Tian, L. Ma, L. Zhang, Y. Hu, J. Wang, M. Wei, D. Yan, D. G. Evans and X. Duan, Adv. Funct. Mater., 2014, 24, 3144…3151.
33 L. Xia, X. Kong, X. Liu, L. Tu, Y. Zhang, Y. Chang, K. Liu, D. Shen, H. Zhao and H. Zhang, Biomaterials, 2014, 35, 4146…4156.
34 J. Liu, W. E. Hennink, M. J. van Steenbergen, R. Zhuo and X. Jiang, J. Mater. Chem. B, 2016, 4, 7022…7030.