Decomposition-assembled porphyrin microparticles with surface protrudent porphyrin nanoparticles for enhanced cellular delivery and photodynamic therapy

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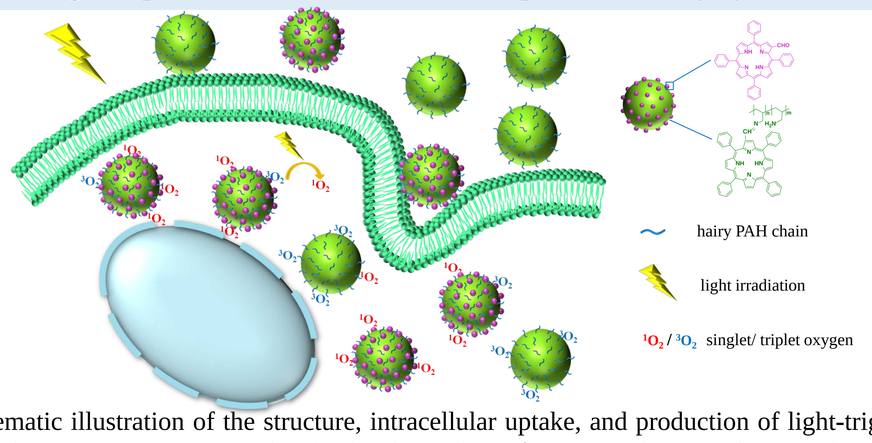
Introduction

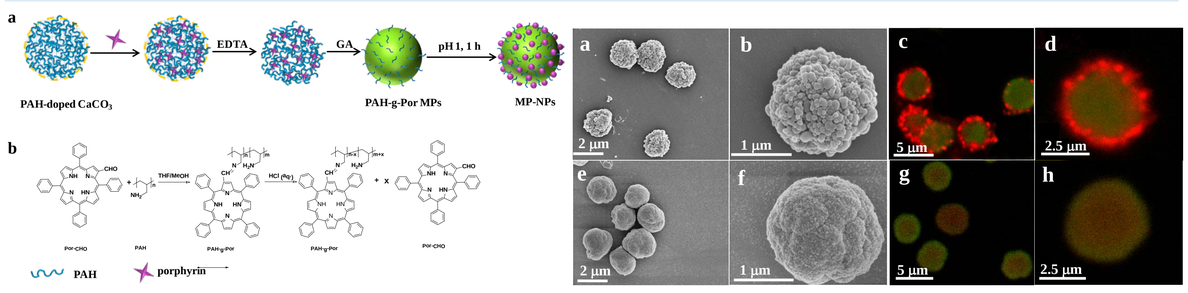
The surface topology of nano/micro particles play a significant role in modulating their interactions with cells, and may serve as a robust strategy for promoting cellular delivery. However, it is still an on-going challenge for the enhanced cellular delivery by surface-engineered organic-based particles. Herein, a novel kind of porphyrin micro-nano particles (MP-NPs), with PAH-g-Por MPs as the core and Por NPs as the protrudent structure on the surface, was prepared through a pH-responsive decomposition-induced assembly method. The Por domains were less aggregated and easily exposed to cells, leading to significantly enhanced cellular uptake, stronger intracellular ROS generation, and more effective photodynamic therapy effect compared with their smooth counterpart microparticles (MPs). The novel concept of turning therapeutics into biomimetic nanomaterials for enhanced cellular delivery and therapy may provide an inspiring horizon for the design of photosensitizer or other therapeutic delivery systems.



Scheme 1. Schematic illustration of the structure, intracellular uptake, and production of light-triggered ROS of the porphyrin micro-nano particles (MP-NPs) with surface protrudent porphyrin NPs and the counterpart smooth MPs. The MP-NPs are superior for internalization, and enhance the production of intracellular ROS, leading to the death of cancer cells.

Materials and Methods

First, poly(allylamine hydrochloride) (PAH)-doped $CaCO_3$ microparticles were reacted with 2-formyl-5,10,15,20-tetraphenylporphyrin (Por-CHO) by Schiff base formation. After template removal with EDTA and crosslinking with glutaraldehyde, the PAH-g-porphyrin microspheres (PAH-g-Por MPs) were obtained. Then the MPs were dispersed and incubated in pH 1 HCl to prepare the micro-nano particles (MP-NPs) (Scheme 2). The obtained MP-NPs and MPs were incubated with HepG2 and A549 cells to research their cellular uptake behaviours and PDT effect (Scheme 1).



Scheme 2 Schematic illustrations of (a) preparation of porphyrin micro-nano particles (MP-NPs) with nano-protrudent surface and the counterpart micro-particles (MPs), and (b) chemical reaction mechanism of formation and decomposition of PAH-g-Por.

Figure 1. Characterization of the MP-NPs (a-d) and (e-h) MPs by SEM (a, b, e, f) and CLSM (c, d, g, h). The MP-NPs were prepared by incubating MPs in pH 1 HCl for 1 h. For CLSM samples, the MPs and MP-NPs were prepared by using larger $CaCO_3$ particles as template. The red fluorescence shows the emission of porphyrin, and the yellow fluorescence is the merged effect of porphyrin and FITC-labeled PAH.

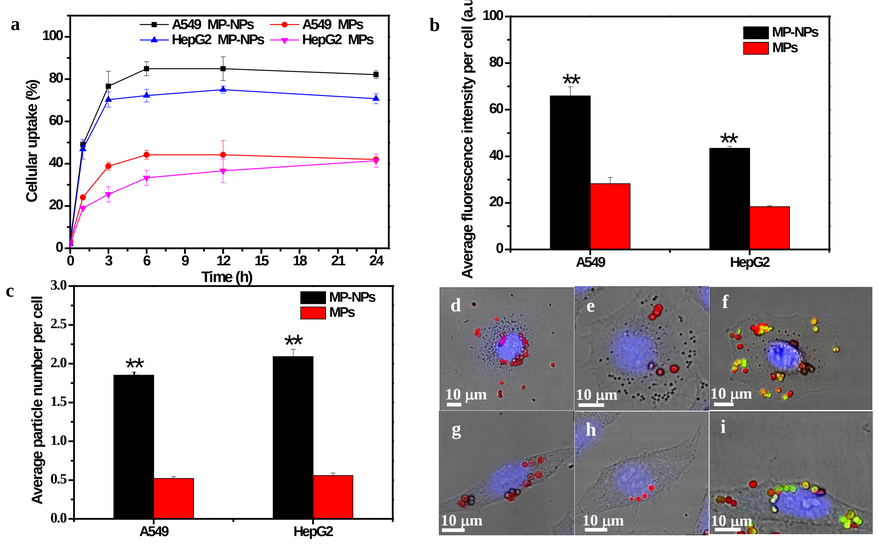


Figure 2 Characterization of cellular uptake of the MP-NPs and MPs. (a, b) Flow cytometry results show the cellular uptake ratio as a function of incubation time (a) and cellular uptake amount of MP-NPs and MPs after being incubated for 3 h (b) at a particle-to-cell ratio of 20: 1. (c) Average number of internalized OGITC-labeled MP-NPs and RBITC-labeled MPs in A549 and HepG2 cells. The particles were co-incubated with cells at a ratio of 10:10:1 (MP-NPs: MPs: cells) for 3 h. (d, e, g, h) CLSM images show the uptake performance of MP-NPs (d, g) and MPs (e, h), which were incubated with A549 (d, e) and HepG2 (g, h) cells at a particle-to-cell ratio of 20: 1 for 3 h, respectively. (f, i) CLSM images show the competitive uptake between OGITC-labeled MP-NPs (green) and RBITC-labeled MPs (red) after co-incubation with A549 (f) and HepG2 (i) cells at a ratio of 10:10:1 (MP-NPs: MPs: cells) for 3 h. (**p < 0.01)

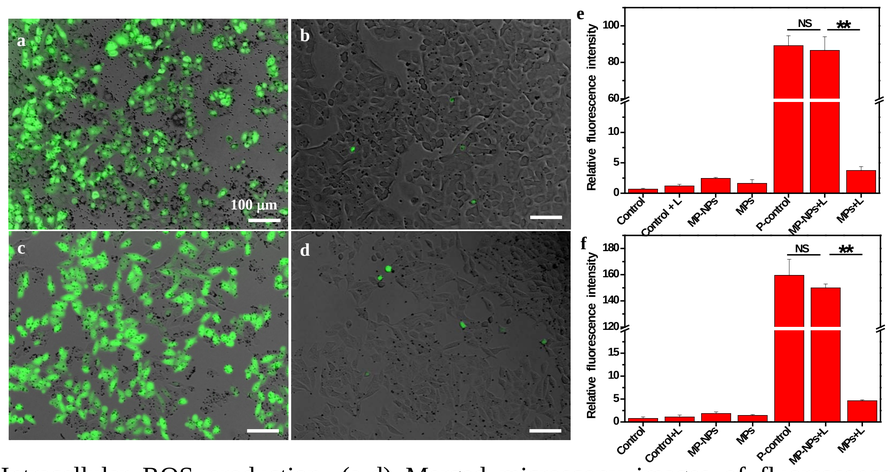


Figure 3. Intracellular ROS production. (a-d) Merged microscopy images of fluorescence mode and transmission mode show the ROS production in A549 cells (a, b) and HepG2 cells (c, d) after incubation with MP-NPs (a, c) and MPs (b, d) for 3 h. The cells were irradiated with laser at 445 nm (0.3 W/cm-2, 5 s), followed by incubation with DCFH-DA for 30 min. Green color represents the existence of ROS. (e, f) The relative concentration of intracellular ROS in A549 (e) and HepG2 (f) cells after different stimulation. The data were obtained by analyzing the fluorescence intensity in microscopy images with Image J software (**p < 0.01, NS indicates no significant difference at p < 0.05).

Results and Discussion

Spherical PAH-g-Por MPs with an average diameter of 1.5 μm were obtained, exhibiting a relatively smooth surface (Figure 1e,f). After being incubated in HCl of pH 1 for 1 h, nanoparticles (NPs) with the size of 105± 10 nm were protruded on the surface of the MPs (Figure 1a,b). In the FITC–labeled MP-NPs, the NPs only showed the red fluorescence of Por but no green fluorescence of PAH-FITC, demonstrating their component was Por (Figure 1c,d,g,h) [3]. After being incubated with HepG2 and A549 cells at a feeding ratio of 20:1, the internalization number and ratio for MP-NPs were more than 2 times higher than those for MPs (Figure 2). The ROS level inside A549 cells treated with MP-NPs was 23.4-fold higher than those treated with MPs, which was comparable to the positive controls. For HepG2 cells, even still higher enhancement (32.6-fold) of ROS generation was observed (Figure 3). Upon illumination with 0.3 W/cm^{-2} light for 5 min, the viability of A549 and HepG2 cells being incubated with the MP-NPs was significantly decreased to 6% and 7%, respectively. However, under the same treatments with MPs, the viability of A549 and HepG2 cells was still at 54% and 43%, respectively (Figure 4). The particularly higher promotion degree of PDT effect of the MP-NPs is mainly contributed by enhanced cellular uptake and improved ROS generation.

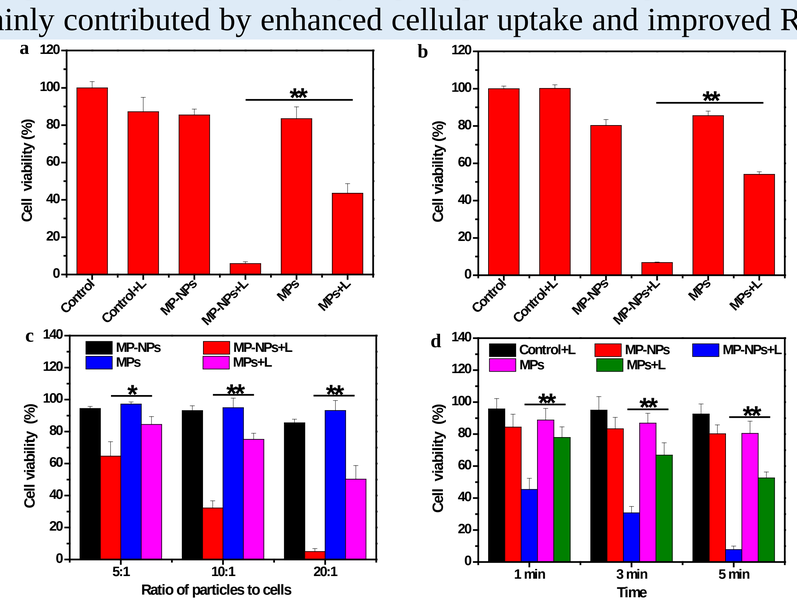


Figure 4. Cell viability after different stimulation. Viability of (a) A549 cells and (b) HepG2 cells after co-incubation with MP-NPs and MPs at a particles-to-cell ratio of 20:1 for 3 h, and then irradiated with or without laser at 445 nm, respectively. (c) Viability of A549 cells after co-incubation with MP-NPs and MPs at different particle-to-cell ratios for 3 h, and then irradiated with or without laser at 445 nm ,respectively. (d) After A549 cells were incubated with MP-NPs and MPs at a particle-to-cell ratio of 20:1 for 3 h, they were irradiated with or without laser at 445 nm for different time. (*p < 0.05, **p < 0.01.)

Conclusion

A novel kind of porphyrin microparticles with surface-protrudent NPs (MP-NPs) resembling the spiky surface of enveloped viruses through a facile pH-responsive self-assembly method. The MP-NPs exhibited high loading efficiency of photosensitizer, and the surface porphyrin NPs promoted cellular uptake with a faster rate and a higher amount than their nanoparticle-free counterparts. The reduced aggregation of porphyrin inside the MP-NPs and the protrudent porphyrin NPs could dramatically enhance ROS generation, leading to very effective PDT effect by combining the improved cellular uptake.

Acknowledgements

This work is financially supported by the Natural Science Foundation of China (21374097 and 21434006), and the 111 Project of China (B16042).

References

[1] Agrawal, M.; Gupta, S.; Stamm, M. J. Mater. Chem. 2011, 21, 615–627
[2] Niu,Y. T.; Yu, M. H.; Yu, C. Z. et al. Adv. Mater. 2013, 25, 6233–6237
[3] Zhang, W. B.; Feng, Y. Q.; Gao, C. Y. et al. Langmuir 2015, 31, 4330−4340