Porphyrin micro-nano particles mimicking viral surface structure for enhanced endocytosis

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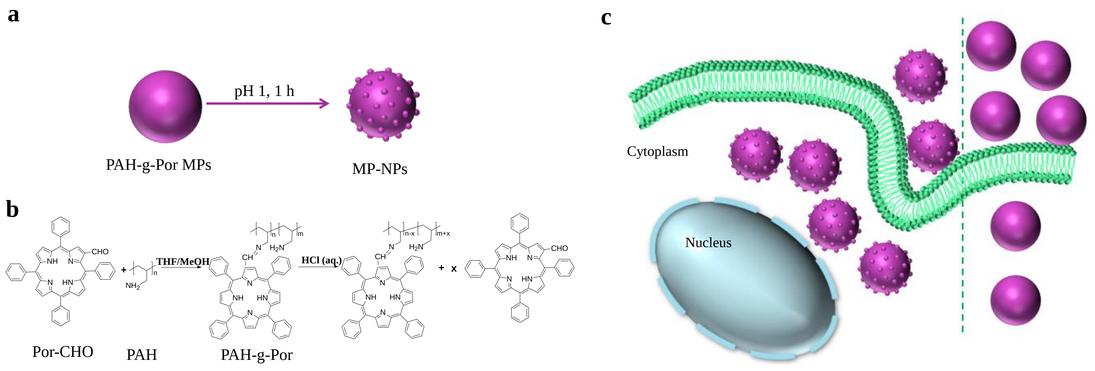
Introduction

Microscopic particles with nanoparticles attached on the surface mimicking the surface morphology of viruses or raspberry have attracted much attention in recent years. However, the preparation methods are limited to electrostatic interaction or emulsion polymerization, most of which usually make use of silica or polystyrene particles as building materials [1]. The surface roughness is utilized to construct superhydrophobic surfaces, stabilize particles or enhance cellular delivery [2]. Yet, mimicking virus surface structure both physically and chemically to enhance endocytosis has rarely been researched so far. Herein, a novel kind of porphyrin micro-nano particles (MP-NPs) is prepared through a newly-developed stimuli-responsive self-assembly method. The NPs on the surface of MP-NPs can be selectively modified with Tat peptide, which is derived from the HIV-1 Tat protein . The enhanced cellular uptake of MP-NPs(Tat) is researched.



Scheme 1. Schematic illustration of (a) preparation of rough porphyrin micro-nano particles (MP-NPs) and smooth micro-particles (MPs) , (b) formation and decomposition of PAH-g-Por, (c) the comparison of cellular uptake performance between MP-NPs and MPs.

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) (Figure 1a). The obtained MP-NPs and MPs were incubated with HepG2 and Hepli cells to research the cellular uptake difference of them (Figure 1c).

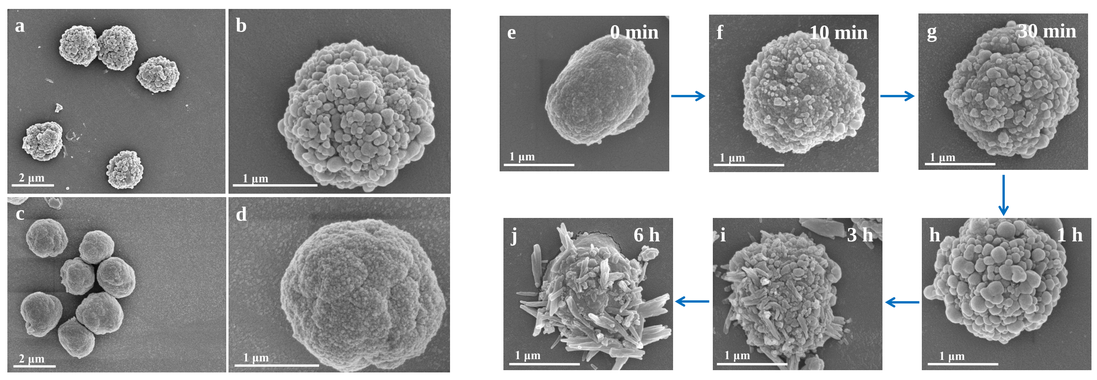
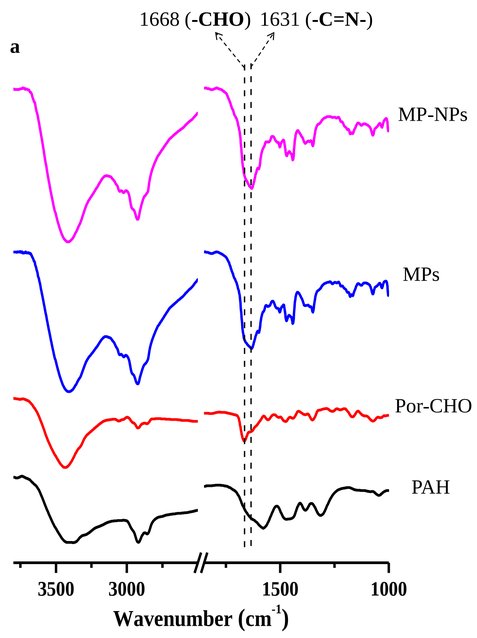


Figure 1. (a-d) SEM images of MP-NPs and MPs, which are prepared by incubating PAH-g-Por microparticles in pH 1 HCl for 1 h (a, b) and 0 min (c, d), respectively. (e-j) SEM images showing the process of nanoparticles protruding on the surface of PAH-g-Por microparticles and turning into nanorods , which are incubated in pH 1 HCl for 0 min (e), 10min (f), 30 min (g), 1 h (h), 3 h (i) and 6 h (g), respectively.



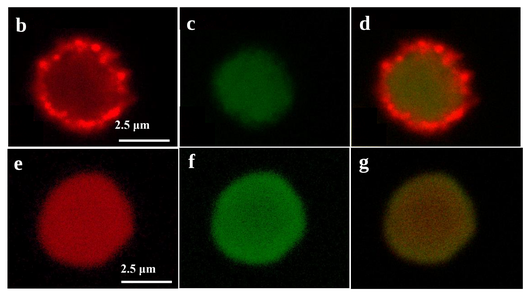


Table 1. Molar ratio of Por to PAH repeat units ([PAH]) calculated according to the elemental analysis results

	MP-NPs	MPs
C/N (w/w)	6.61	5.99
Por: [PAH] (mol%)	19.9	12.3

Figure 2.(a) FTIR spectra of MP-NPs and MPs. (b-g) CLSM images of MP-NPs (b-d) and MPs (e-g) prepared by using larger $CaCO_3$ as templates; The red (b, e) and green (e, f) fluorescence show the emission of porphyrin and PAH (FITC labeled), respectively; (d) Merged image of (b) and (c); (g) Merged image of (e) and (f).

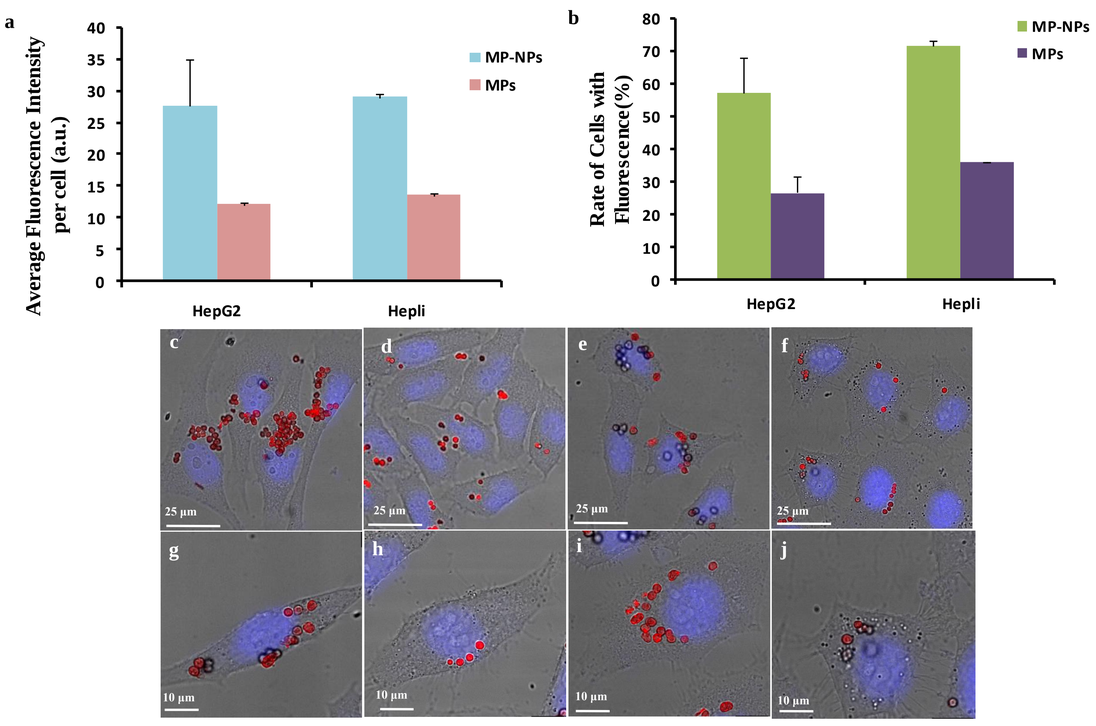


Figure 3. (a, b) Flow cytometry results showing the endocytosis amount (a) and rate (b) difference between MP-NPs and MPs. (c-j) CLSM images showing the endocytosis difference between MP-NPs (c, e, g, i) and MPs (d, f, h, j), which are incubated with HepG2 (c, d, g, h) and Hepli (e, f, i, j) cells for 12 h.

Results and Discussion

Spherical PAH-g-Por MPs with an average diameter of 1.5 μm were obtained, exhibiting a relatively smooth surface (Figure 1c, d). After being incubated in HCl of pH 1, nanoparticles (NPs) were protruded on the surface of the MPs. The number and size of NPs increased with extension of time within 1 h and turned into nanorods after 1 h (Figure 1e-j). At 1 h, the size of NPs became 105± 10 nm (Figure 1a, b). MP-NPs and MPs had similar chemical components (Figure 2a, Table 1). 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 2b-g) [3]. After being incubated with HepG2 and Hepli cells for 12 h at a feeding ratio of 20:1, the endocytosis amount and rate of MP-NPs were more than 2 times higher than those of MPs (Figure 3). After modification with Tat peptide, the endocytosis amount and rate of MP-NPs were increased by 22% and 27%, respectively, while there was no obvious increase for MPs (Figure 4). The results demonstrate the selective modification of NPs on the surface of MP-NPs.

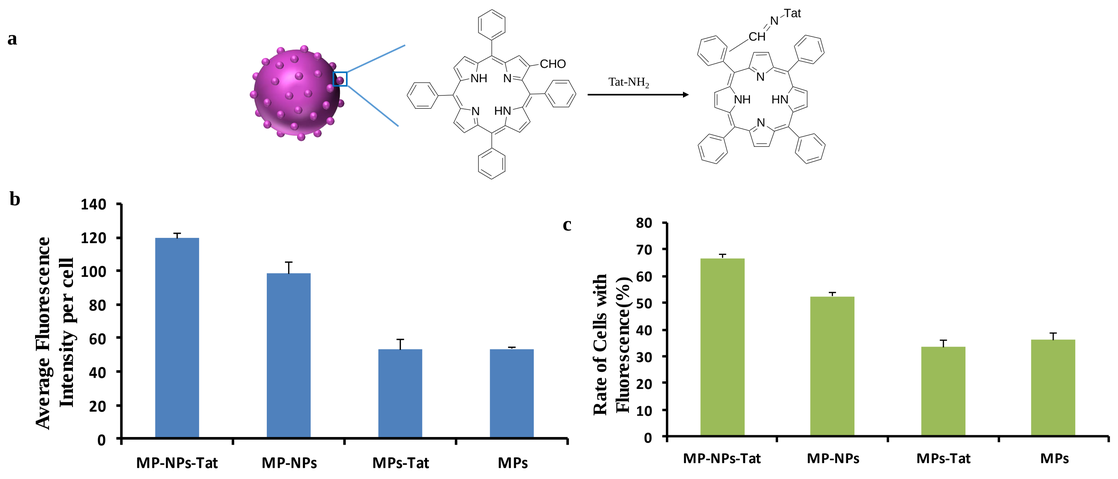


Figure 4. (a) Schematic illustration of selectively modification of NPs with Tat peptide on the surface of MP-NPs; (b, c) Flow cytometry results showing the endocytosis amount (b) and rate (c) difference between MP-NPs-Tat, MP-NPs, MPs-Tat and MPs, which are incubated with HepG2 cells for 12 h.

Conclusion

A novel kind of porphyrin micro-nano particles (MP-NPs) mimicking the surface structure of viruses is prepared with a pH-responsive self-assembly method. The formation of MP-NPs results from the decomposition of Schiff base and self-assembly of Por. The NPs on the surface of MP-NPs can be selectively modified with Tat peptide. Both the roughness and Tat modification of MP-NPs can enhance the cellular uptake. The MP-NPs may be utilized for cell delivery and cancer therapy, such as photodynamic therapy.

Acknowledgements

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References

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