Tailoring the pH-induced aggregation of Mixed-charge Gold Nanoparticles in different sizes for Photothermal Therapy

Huan Li, Xiangsheng Liu, Nan Huang, Qiao Jin, Kefeng Ren and Jian Ji*

Department of Polymer Science, Key Laboratory of Macromolecule Synthesis and Functionalzation of Minster of Education , Zhejiang University, Hangzhou, 310027, China

***Contact to: Ji Jian, email: jijian@zju.edu.cn, Tel/Fax: +86-571-87953729**

Introduction

Photothermal therapy, collaborating with the near-infrared (NIR) light, can be used to treat solid tumors in a minimally invasive way, which brings new hope to cancer therapy. Application of spherical gold nanoparticles in photothermal therapy has been hindered because of the insufficient NIR light absorption. When nanospheres forming aggregates[1], there is a red-shift in the light absorption spectra that sometimes can even attain to the NIR region. Mixed-charge gold nanoparticles (MC-GNPs) [2, 3] have shown tunable pH-sensitive aggregation behaviors, which combining with the aggregation -induced red-shift in light absorption may enable MC-GNPs to be applied in photothermal therapy. In this research, we systematically studied how the pH-induced aggregation behaviors of MC-GNPs can be tailored by the surface ligand composition and nanoparticle size. Then, the photothermal therapy efficacy was evaluated to pave the way for further appication.

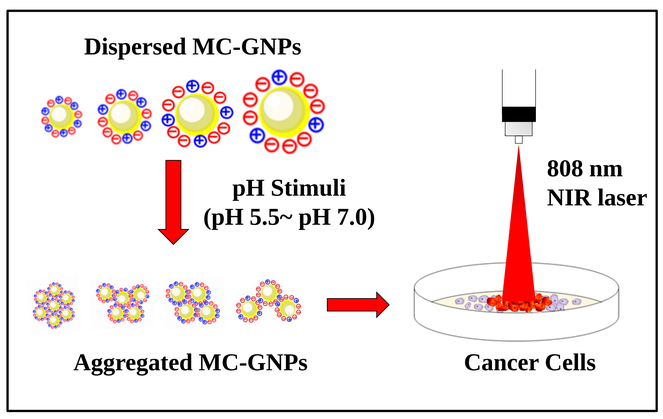

Fig. 1. Schematic illustration of MC-GNPs responding to tumor acidic pH to be used in photothermal therapy by pH-induced aggregation.

Method

We prepared pH-sensitive mixed-charge zwitterionic AuNPs in four different sizes. Then they were modified with mixed SAMs of weak electrolytic 11-mercaptoundecanoic acid (MUA) and strong electrolytic (10-mercaptodecyl) trimethyl-ammonium bromide (TMA).[3] The pH at which the well resolved nanoparticles form aggregates can be tuned by changing the feed ratio of the two ligands, that is, increasing the proportion of MUA leading to a decrease in aggregation pH. With trial and error, an optimum feed ratio of the two ligands for the four different size gold nanoparticles to show abrupt aggregation at tumor acidic pH were found. Then, the photothermal efficacy of those MC-GNPs were further examined.

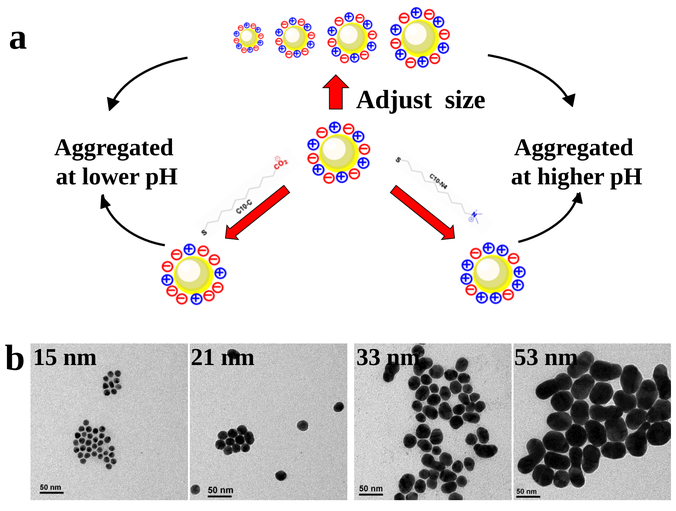

Fig. 2. (a) Schematic illustration of tailoring pH-induced aggregation of MC-GNPs by adjusting size or surface ligand proportion. (b) TEM images of different size MC-GNPs with ligand feed ratio = 1.

Results and discussion

pH sensitivities of MC-GNPs with feed ratio = 1

With the same ligand feed ratio, the MC-GNPs in different sizes showed diverse pH-induced aggregation behaviours. Only those MC-GNPs with the size of 15 nm exhibited effective pH-sensitivity to the tumor acidic pH. Further tailoring of surface ligand composition was required to attain MC-GNPs that aggregate abruptly at pH 7.0 ~ pH 6.8.

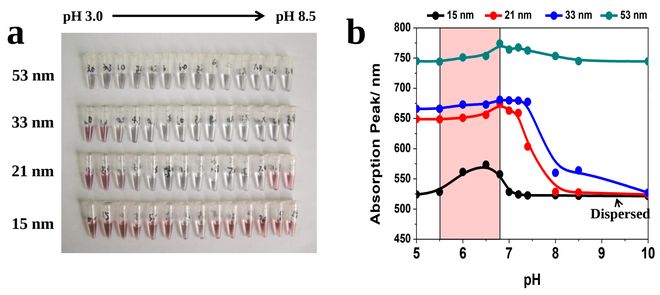

Fig. 3. (a) Aggregation behaviours of the MC-GNPs in different sizes with the same ligand feed ratio. (b) Absorbance peak values of MC-GNPs in different sizes detected by UV-vis spectrum once added to PBS (phosphate buffered sailine, 50 mM) of different pH values. The pink window represents the pH range of tumor acidic environment.

Tailoring pH-induced aggregation of MC-GNPs

By increasing the proportion of MUA in the SAMs, MC-GNPs which aggregated at lower pH were attained. With proper control on surface ligand composition, MC-GNPs with desired pH-induced aggregation behaviors can be prepared.

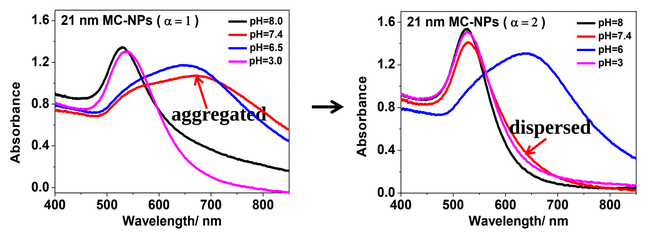

Fig. 4. Contrast of pH-induced aggregation behaviour of 21 nm MC-GNPs with different surface ligand feed ratio. α is the molar feed ratio of MUA against TMA, namely $\alpha = M_{MUA}/M_{TMA}$.

Different size MC-GNPs respond to tumor acidic pH.

With proper adjustment, those four different size MC-GNPs can all exhibit desired pH-induced aggregation at the tumor acidic pH. The same phenomenon were also observed in pH 6.5 cell culture media, which allowed further analysis of photothermal therapy efficacy *in vitro*.

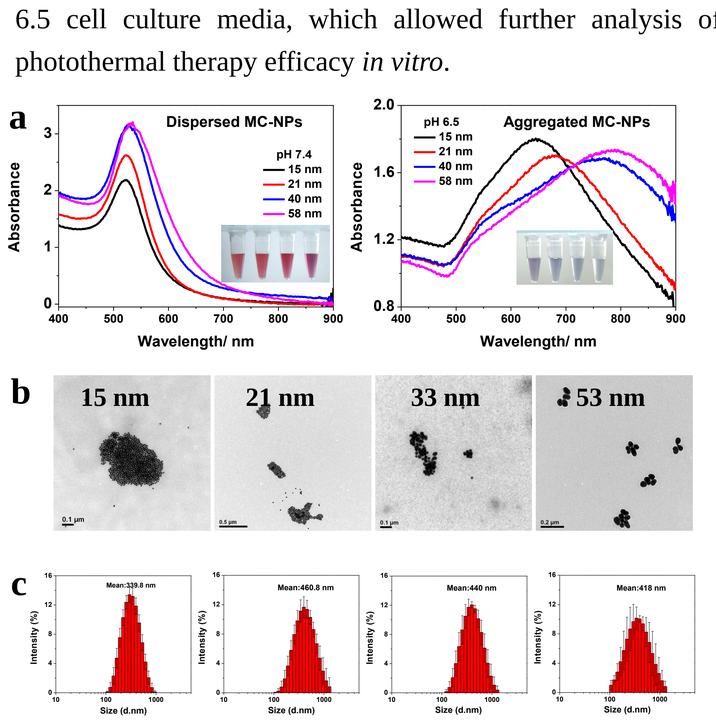

Fig. 5. MC-GNPs that can respond to tumor acidic pH stimuli. (a) UV-vis spectra of dispersed MC-GNPs and aggregated MC-GNPs in pH 7.4 PBS and pH 6.5 PBS, respectively. Insets are corresponding digital images. (b)TEM images of aggregated MC-GNPs in different sizes, and (c) their size distribution measured by DLS.

Photothermal therapy efficacy of MC-GNPs

The NIR light-heat converting ability of MC-GNPs was inspiring. As the size increased, MC-GNPs generated more heat with the 808 nm laser irradiation. After multiple irradiation, the heat generating ability of MC-GNPs remained the same. The HepG2 cancer cells cultured with MC-GNPs aggregates could be efficiently killed by the 808 nm laser irradiation. Among others, the largest 53 nm MC-GNPs showed the best photothermal therapy efficacy.

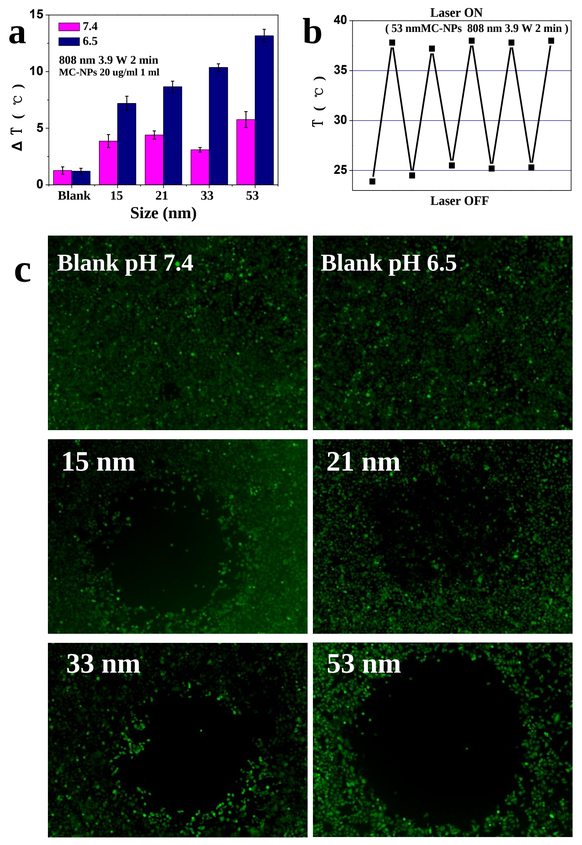

Fig. 6. Photothermal therapy efficacy of MC-GNPs. (a) Temperature rise of different size MC-GNPs solution after the NIR laser irradiation. (b) Multiple irradiation of 53 nm MC-GNPs solution by the NIR laser. (c) HepG2 cells incubated with different size MC-GNPs and irradiated by the NIR laser. Cells were stained with FDA. Control group were incubated with pH 7.4 and pH 6.5 normal cell culture media.

Conclusion

In summary, we systematically explored the basic law of the pH-induced aggregation of MC-GNPs influenced by surface charge composition and size factors. Given this, we can synthesize MC-GNPs with desired pH-induced aggregation behaviours. In this study, we prepared four different size MC-GNPs that all respond to tumor acidic pH. The aggregates of MC-GNPs, especially from those 53 nm ones, showed encouraging photothermal therapy efficacy *in vitro* which brings about new horizon to photothermal cancer therapy.

Acknowledgements

Financial supports from the National Science Fund for Distinguished Young Scholars (51025312), the National Basic Research Program of China (2011CB606203), NSFC-50830106, 21174126 and Open Project of State Key Laboratory of Supramolecular Structure and Materials (SKLSSM 201204) are gratefully acknowledged.

References

1. Nam, J. et al., *J. Am. Chem. Soc.* **2009**, *131*, 13639.
2. Pillai, P. P. et al., *J. Am. Chem. Soc.* **2013**, *135*, 6392.
3. Liu, X. et al., *ACS Nano* **2013**, 7: 6244.