Preparation of TAT-modified PNIPAM microgel particles and their cellular uptake, intracellular distribution, and influence on cytoviability in response to temperature

Wenjing Zhang, Zhengwei Mao*; Changyou Gao*

MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

* E-mail: zwmao@zju.edu.cn; cygao@zju.edu.cn

Introduction

Thermo-sensitive microgel particles may exert a swelling force inside cells and influence on cell viability due to their volume transition in response to external temperature change[1, 2]. In this study, we investigated the influence of PNIPAM microgel particles uptake on cell viability in response to external temperature change.

Results

In this study, cross-linked poly(N-isopropylacrylamide) (PNIPAM) microgel particles with a thermo-responsive volume expansion ability were prepared by precipitation polymerization of NIPAM, poly(ethylene glycol) diacrylate and acrylic acid. Cell penetrating peptide TAT was modified to endow the microgel particles with enhanced cellular uptake.

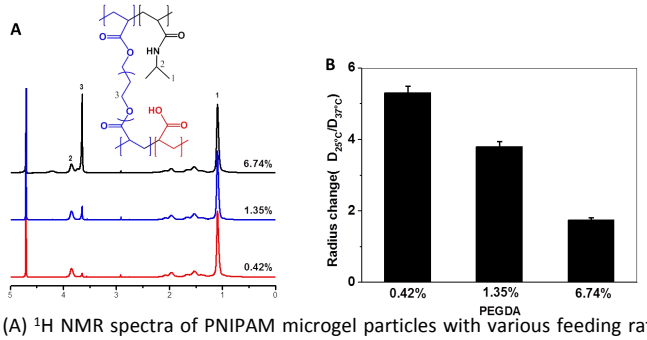

Figure 2. (A) ^{1}H NMR spectra of PNIPAM microgel particles with various feeding ratios of PEGDA noted in the figure. (B) The ratio of particle radius under 25 °C to that under 37 °C as a function of PEGDA content.

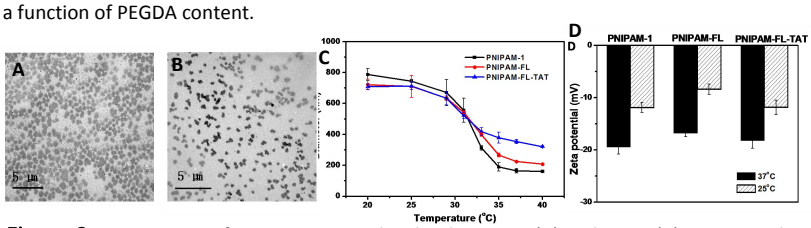

Figure 3. TEM images of PNIPAM-1 particles dried at 25 °C (A) and 37 °C (B), respectively. (C) Diameter of PNIPAM-1, PNIPAM-FL and PNIPAM-FL-TAT particles in cell culture medium as a function of temperature. (D) Zeta potential of various PNIPAM particles measured at 25 °C and 37 °C in cell culture medium, respectively.

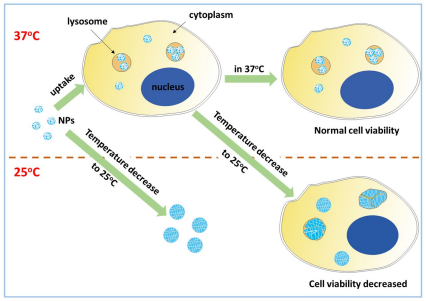

Figure 1. A schematic illustration showing uptake of temperature responsive particles, and particles expand inside and outside of cell.

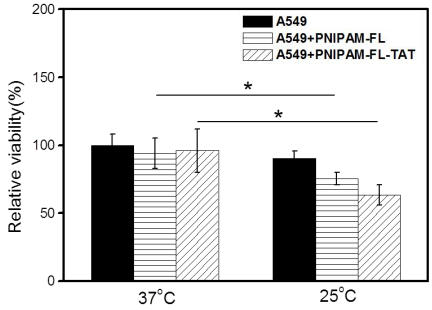

Figure 4. Influence of temperature change on cell viability after being incubated with PNIPAM-FL and PNIPAM-FL-TAT particles. The cells were co-cultured with 200 μ g/mL PNIPAM-FL and PNIPAM-FL-TAT particles for 24 h at 37 °C, and then cultured at 25 °C or 37 °C for 4 h, respectively. Another culture at 37 °C was carried out for 24 h before the cell viability assay. Particle-free cells were used as a control.

The cellular uptake, intracellular distribution and thermo-responsive cytotoxicity of the microgel particles were studied by co-culture with lung adenocarcinoma (A549) cells. The PNIAPM microgel particles were largely ingested by A549 cells and mainly located in lysosomes. TAT modification enhanced the cellular internalization of particles but did not alter their intracellular distribution. While the PNIPAM microgel particles did not show significant impact on cell viability at 37 °C, they caused cytotoxicity to some extent when being cultured at 25 °C for 4 h.

Acknowledgements

Financially supported by the Natural Science Foundation of China (51120135001, 2011CB606203).

References

[1] Zhang W., Mao, Z., & Gao, C. (2014). *Journal of Colloid and Interface Science*, 15(434), 122–129.
[2]Zhang, Y., Hu, L., Yu, D., & Gao, C. (2010). *Biomaterials*, 31(32), 8465–74.