Ultra Long-Term Cellular Tracing by Fluorescent AIE Bioconjugate with Good Water Solubility over a Wide pH Range

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Abstract: **The aggregation-caused quenching effect of conventional organic dyes and the high toxicity of inorganic quantum dots have been thorny issues that have constantly obsessed scientists in the area of research. Alarge number of tetraphenylethene (TPE) units are successfully attached to N-succinyl-chitosan (NSCS) macromolecular chains to fabricate a novel TPE-NSCS fluorescent bioconjugate, which could be strongly emissive in the solid state due to its aggregation-induced emission effect. TPE-NSCS can well be solubilized in water over a wide range of pH values. We were surprised to see that the stained cells still showed a bright fluorescence emission for as many as 30 passages. The water-solubility over a wide pH range, ultra long-term retention in cells, and strong fluorescent signals indicate TPE-NSCS is one promising candidate for various biomedical applications.**

Introduction

In modern biomedical research, *in vitro/in vivo* cellular tracing over a long period of time can offer significant information on a variety of intricate biological processes during pathological and physiological research, including carcinoma development, cancer treatment, cell therapies, immune responses, and so on.

In the past decade, AIE fluorescent probe were explored for cell imaging and cell tracing. Nevertheless, taking into account the complexity of the cell/tissue and the time-requirement for long-term tracing, previous investigations displayed some flaws, such as a fluorogen transition to the daughter cells at just around 10 generations; and the previous cell tracing AIE probes with aromatic nucleus could not well be dissolved in aqueous solutions over a wide pH range which limited their further biomedical applications.

Herein, we attach a large number of TPE units to NSCS chains to prepare a TPE-NSCS fluorescent AIE probe, which can be solubilized in water across a wide pH range. The highly emissive AIE bioconjugate can be internalized by living HeLa cells, and traced for 30 passages, which is superior to any previous reports

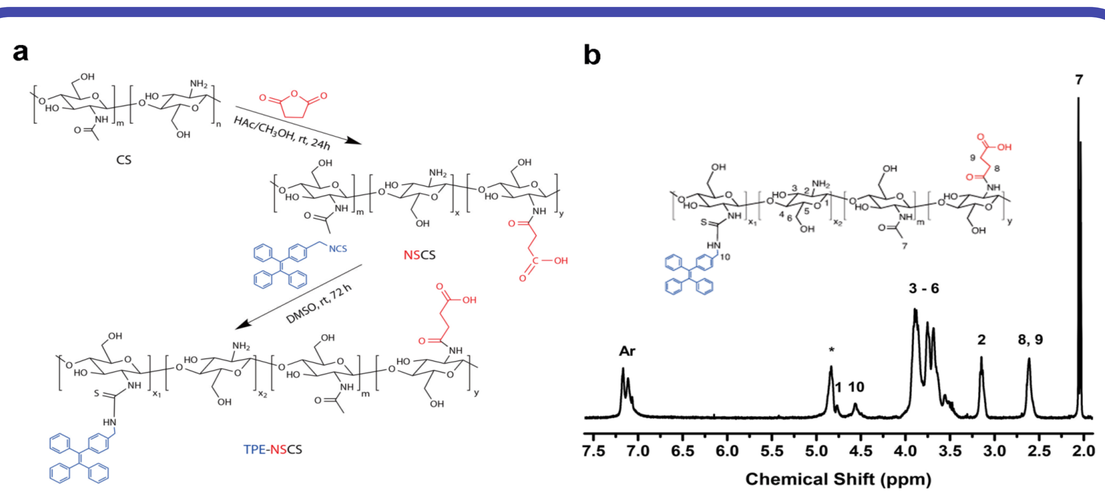

Fig. 1. Synthesis of TPE-NSCS. (a) Scheme for the TPE-NSCS synthetic route, (b) ^{1}H NMR spectrum for TPE-NSCS.

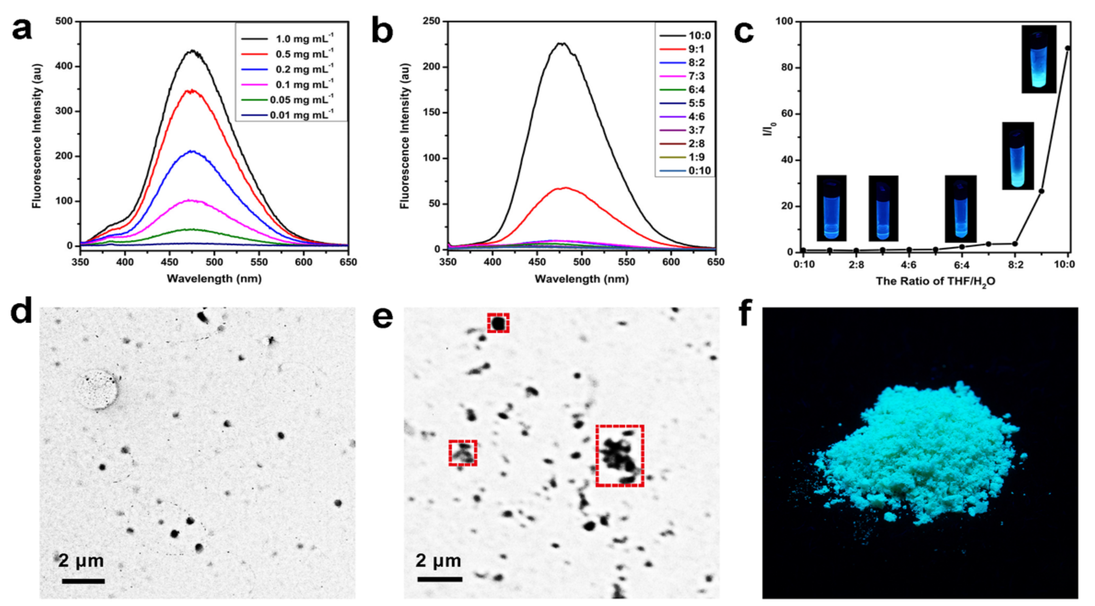

Fig.2. AIE effect of TPE-NSCS. (a) Fluorescence spectra of the TPE-NSCS aqueous solution at different concentrations; (b) Fluorescence spectra of the TPE-NSCS solution (0.01 mg mL-1) at different THF/H2O ratios; (c) The relationship between I/I0 and the ratio of THF/H2O, where I0 and I are the fluorescence intensities of TPE-NSCS in the absence and presence of THF, respectively. Insets are photographs of the TPE-NSCS solutions excited by UV light; (d, e) TEM images of the TPE-NSCS aqueous solution with concentrations of (d) 0.2 mg mL-1 and (e) 2 mg mL-1, respectively; (f) Photograph of TPE-NSCS solid powder taken under UV illumination.

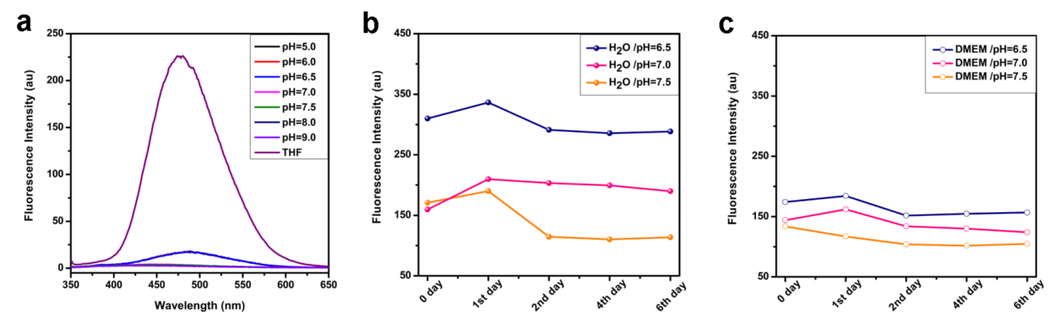

Fig. 3. Water solubility of TPE-NSCS. (a) Fluorescence spectra of the TPE-NSCS aqueous solution (0.01 mg mL^{-1}) at different pH values; (b, c) Fluorescence intensity (480 nm) of TPE-NSCS (0.2 mg mL^{-1}) in (b) an aqueous solution and (c) a DMEM solution allowed to sit for 6 days.

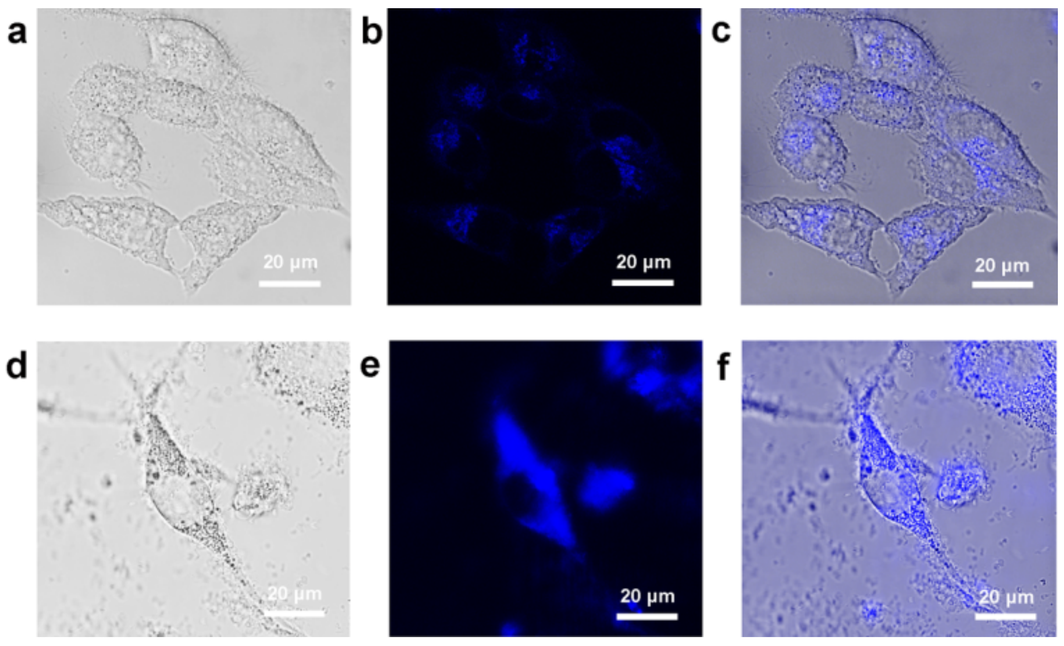

Fig. 4. HeLa cells stained by TPE-NSCS for different time. CLSM images of HeLa cells incubated with 0.2 mg mL^{-1} TPE-NSCS, stained for (a–c) 6 h, (d–f) 24 h. (a, d) bright field, (b, e) excited with 405 nm laser, and (c, f) merged images. Scale bar=20 μm.

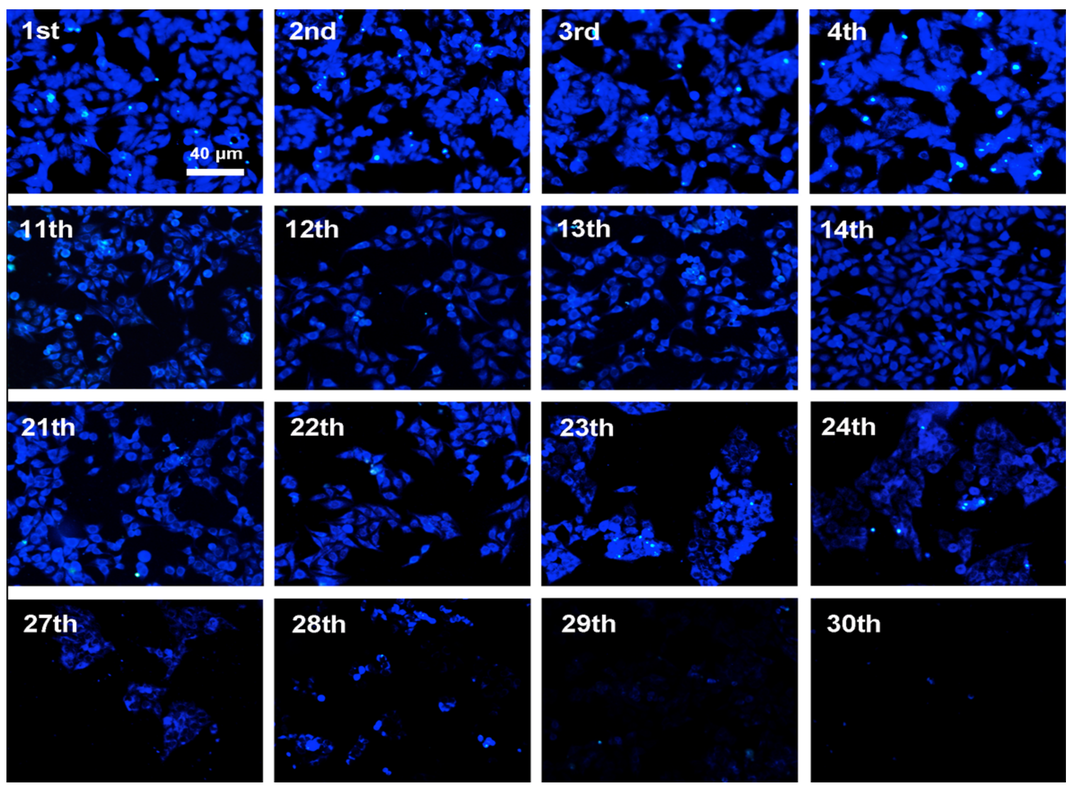

Fig. 5. Ultra long-term cellular tracing. HeLa cells traced by TPE-NSCS (0.2 mg mL^{-1}) at different passages. Scale bar=40 μm.

Conclusions

We successfully designed and synthesized a TPE-NSCS adduct with the AIE characteristic by attaching TPE units to NSCS macromolecular chains. The AIE effect makes the TPE-NSCS bioprobe simple to use and easy to handle in operational procedures. TPE-NSCS can well be solubilized in water over a wide range of pH values. The fluorescent TPE-NSCS bioconjugate has good cytocompatibility, which can strongly bind the whole cell cytoplasmic regions, thus realizing ultra long-term cellular tracing for 30 passages. The water-solubility over a wide pH range, ultra long-term retention in cells, and strong fluorescent signals indicate TPE-NSCS is outstanding for various biomedical applications as a fluorescent probe.

Acknowledgement

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References

1. Z. Wang, L. Yang, Y. Liu, X, Huang, F, Qiao, W. Qin, Q. Hu and B. Tang, *Journal of Materials Chemistry B*, 2017.