Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Materials

Supplementary Table

Table S1 Integration information from the ^{1}H NMR spectrum of TPE-NSCS.

Protons of different units	δ (ppm)	Integration	Relative amount of units
-C<u>H</u>NH_2	2.9-3.1	1	1/1
-CH_2CH_2-	2.5-2.7	1.83	1.83/4
Ar	7.0-7.25	1.3205	1.3205/19

Setting the total repeating sugar ring units in chitosan as 100%; degree of substitution (D.S.) values and degree of labeling (D.L.) values were calculated based on the data shown in Table S1 using the following equations:

D.S.=[(1.83/4)/(1/1)]*100%=45.75%

D.L.=[(1.3205/19)/(1/1)] *100%=6.95%

Supplementary Figure

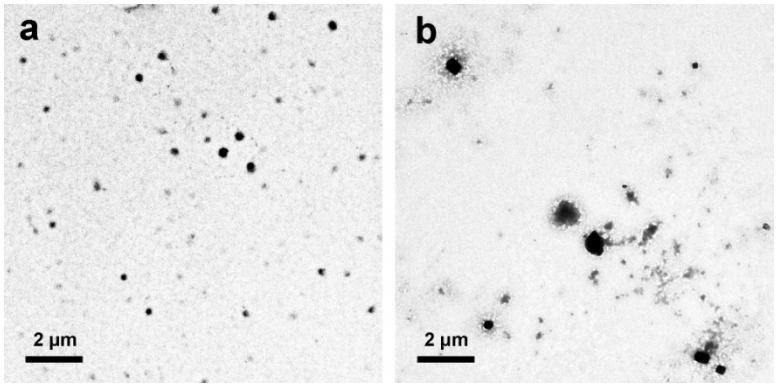

Fig. S1 TEM images of TPE-NSCS in different solvents. (a) in neutral aqueous solution; (b) in THF.

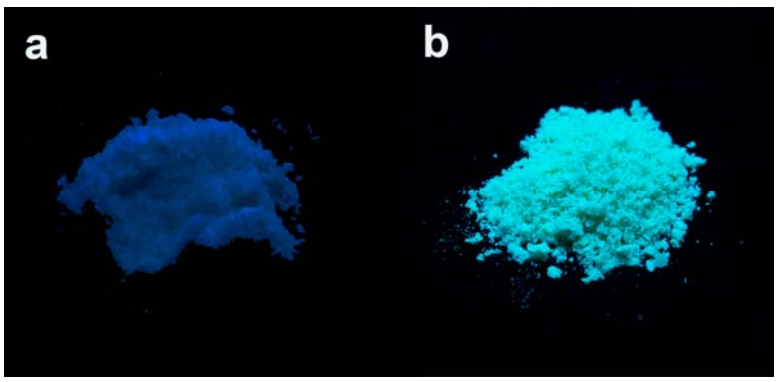

Fig. S2 Photograph of TPE-NSCS solid powder taken under UV illumination with degree of labeling: (a) 0.85%

and (b) 6.95%, respectively.

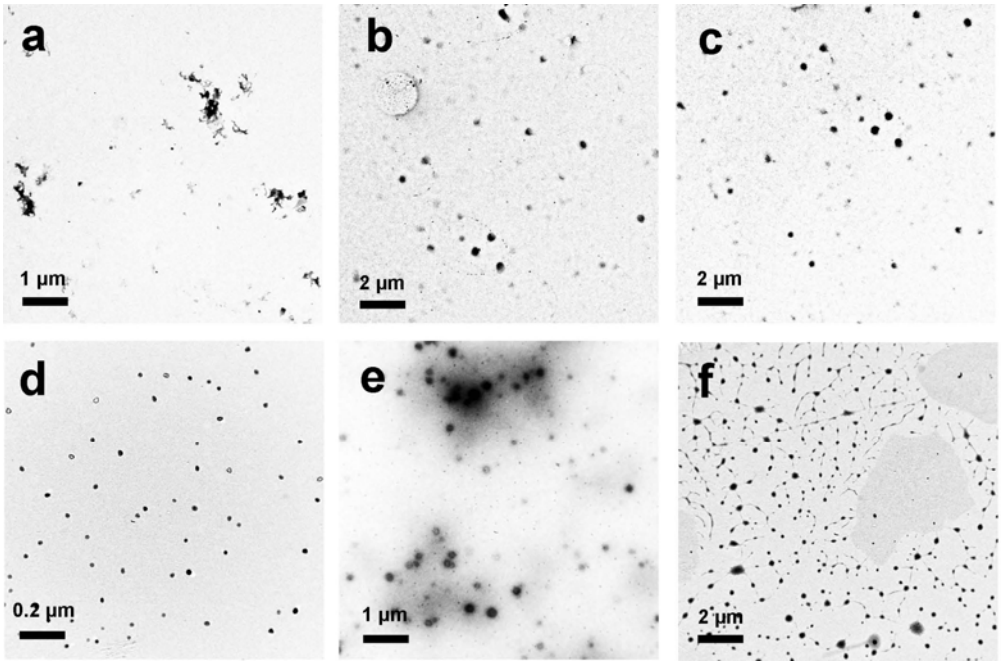

Fig. S3 TEM images of TPE-NSCS. (a–c) aqueous solution with pH values of 6.5, 7, and 7.5, respectively; (d–f) DMEM solution with pH values of 6.5, 7, and 7.5, respectively.

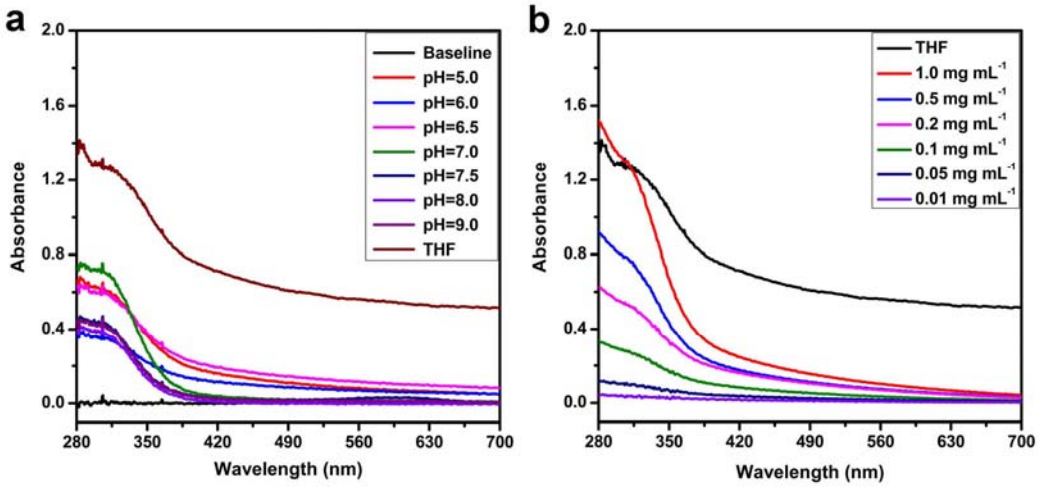

Fig. S4 UV-vis spectroscopy of TPE-NSCS. (a) with different pH values in water or THF; (b) with different concentrations in water or THF.

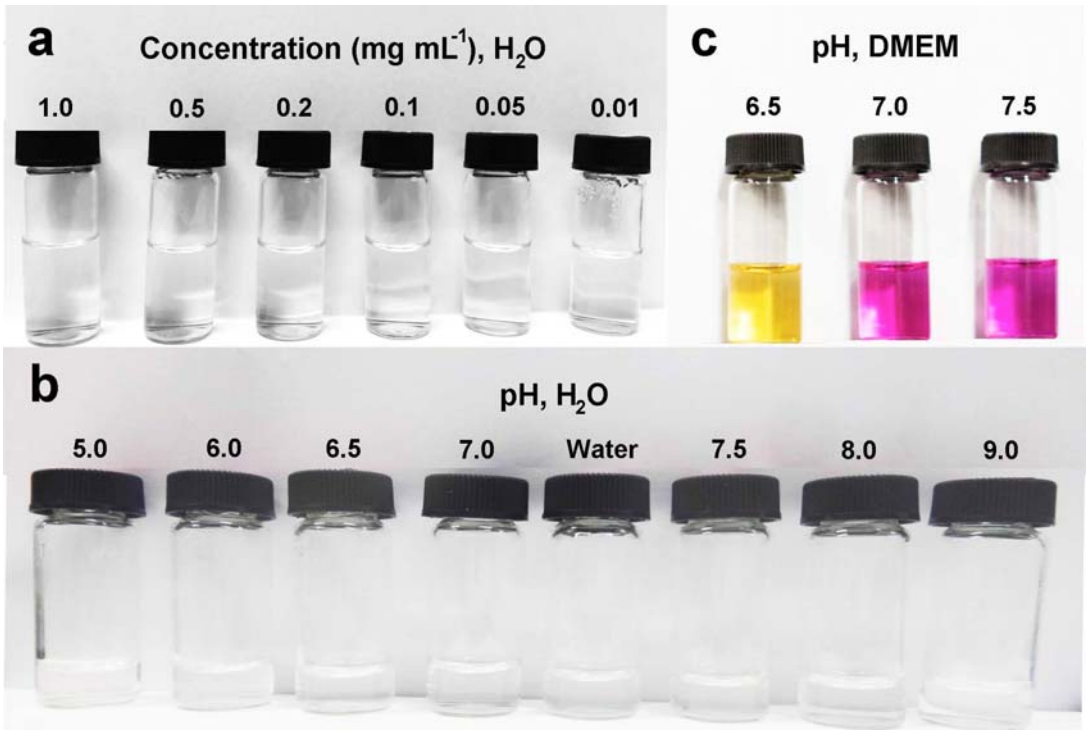

Fig. S5 Photographs of the TPE-NSCS solution. (a) aqueous solution with different concentrations; (b) aqueous solution with different pH values; (c) TPE-NSCS DMEM solution with different pH values.

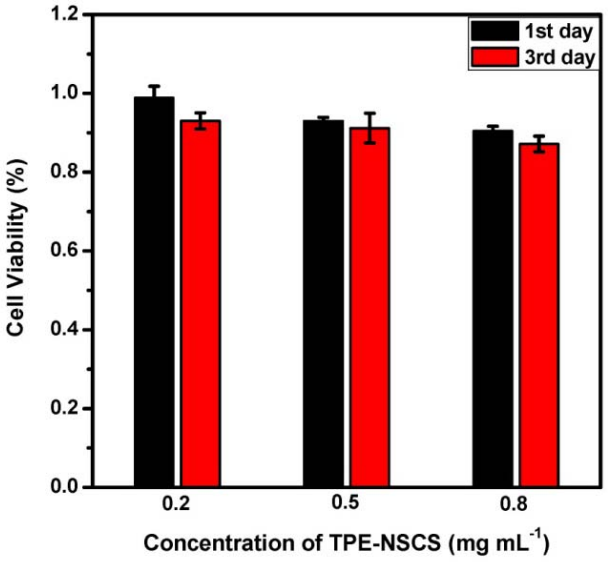

Fig. S6 Cytotoxicity of TPE-NSCS. Cell viability of TPE-NSCS toward the HeLa cells by MTT assay. Triplicate experiments were performed each time.

Supplementary Discussion

Hydrophilic –COOH and $–NH_2$ groups endow the TPE-NSCS bioconjugate with an amphiphilic

polyelectrolyte characteristic, which is a polycation in an acid solution, while it turns into a polyanion in an alkaline solution. In addition, the fluorogen TPE units are hydrophobic, so TPE-NSCS can self-assemble into spherical nanoparticles due to the hydrophobic–hydrophilic interactions. TPE-NSCS solubilized in an acid solution showed a random flocculent state with a size of more than 1 μm, while forming uniform nanoparticles in neutral and alkaline solutions with diameters around 400 nm and 80 nm (Supplementary Fig.3).

The samples with pH 7 and 7.5 kept their original morphologies and almost no change occurred after being mixed with the culture medium (Supplementary Fig.3). However, the TPE-NSCS DMEM solution at pH 6.5 changed to uniform nanoparticles (about 50 nm), which is obviously different from the TPE-NSCS aqueous solution at the same pH value with the flocculent structure (more than 1 μm). An acid solution may protonate the amino groups in a CS chain, which causes a combination with the protein macromolecules in a culture medium, and grants the nanoparticles a uniform size.

The water solubility of TPE-NSCS was further demonstrated by UV-vis spectrophotometry. In general, a turbid solution can lead to an overall upshift of the absorbance curve in the visible spectrum from 400 nm to 600 nm, while the absorbance of a clear solution remains close to the baseline in the visible range.[1] Since TPE-NSCS exhibits good water solubility over a wide pH range, it is expected that there is no or a little upshift compared with the baseline as the pH of the aqueous solution is changed. Consistent with this speculation, the visible spectra showed some faint upshift in the acid solution, while it showed hardly any upshift in the neutral and alkaline solutions (Supplementary Fig.4). Meanwhile, the UV-vis spectra revealed the differences in the TPE-NSCS solubilized in acid, neutral, and alkaline solutions once again, which could well explain the fluorescence intensity variation at different pH values. We also provided photographs of the TPE-NSCS aqueous solution in H_2O and DMEM over a wide pH range (Supplementary Fig.5), which were directly observed to be clear and transparent. These results confirmed the good water solubility of TPE-NSCS over a wide range of pH values.

References

1 K. Kim, J.H. Ryu, D.Y. Lee and H. Lee, *Biomaterials Science*, 2013, **1**, 783-790.