

Supporting Information

Size-Controlled, Colloidally Stable and Functional Nanoparticles Based on Molecular Assembly of Green Tea Polyphenols and Keratins for Cancer Therapy

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Experimental Section

Extraction of Hair Proteins

Human hair (of a barber shop giving) was washed with water; external lipids were removed using a mixture of chloroform/methanol (2:1, v/v) for 24 h. The delipidized hair (10 g) was mixed with a solution (500 mL) containing 5 g SDS, 25mM Tris-HCl, pH 8.5, 2.6 M thiourea, 5 M urea and 5% 2-mercaptoethanol (2-ME) at 50 °C for 1—3 d. The mixture was filtered and centrifuged at 10000 r/min for 10 min at room temperature. The obtained supernatant was collected as a hair protein fraction.

Preparation of oligomeric catechins (OCat)

The oligomeric catechins (OCat) were synthesized by one step. Catechins (0.5 g), HCHO (1 mL), and acetic acid (5 mL) were added into flask, then stirred at room

temperature for 12 h. The oligomeric catechins collected by centrifuge. After freeze drying, the precipitates were further characterized and analyzed.

SDS Polyacrylamide Gel Electrophoresis.

SDS-PAGE analysis was used to determine the molecular weight of keratin. Extracted keratin solutions were diluted to 1 mg/mL with deionized water and mixed 1:1 with 2x SDS loading sample buffer containing 5% 2-mercaptoethanol. The proteins (0.2 mL samples) were loaded onto precast 10% gradient Tris-HCl gels. Separation was performed at 100 V for 90 min. After separation, gel was stained with Coomassie brilliant blue (G250), 10% acetic acid, and 50% methanol for 1 h and destained in 10% acetic acid and 45% ethanol. Samples were compared to a standard ladder (Precision Plus Protein™ Standards, Bio-Rad) and the gel was imaged with a GelDoc 2000 System (Bio-Rad).

Table S1. Key parameters of nanoparticles np-1 - np-9.

| Sample | Keratin (mg/mL) | Catechin (EGCG≥80%) (mg/mL) | HCHO (mol/L) | Diameter* (nm) | productivity |
|---------------|----------------------------|--|-------------------------|---------------------------|---------------------|
| np-1 | 0.02 | 5 | 0.1 | 232 | 67.3% |
| np-2 | 0.1 | 5 | 0.1 | 198 | 63.5% |
| np-3 | 0.2 | 5 | 0.1 | 158 | 63% |
| np-4 | 0.1 | 5 | 0.05 | 86 | 35.3% |
| np-5 | 0.1 | 5 | 0.02 | 67 | 18.6% |
| np-6 | 0.1 | 5 | 0.004 | 31 | 7.8% |
| np-7 | 0.1 | 2.5 | 0.1 | 76 | 41.5% |
| np-8 | 0.1 | 1 | 0.1 | 43 | 21.2% |
| np-9 | 0.1 | 0.5 | 0.1 | 30 | 6.4% |
| kf-0 | 0.1 | - | 0.1 | clear solution | |
| kc-0 | 0.1 | 5 | - | clear solution | |

| | | | | |
|------|---|---|-----|----------------|
| cf-0 | - | 5 | 0.1 | clear solution |
|------|---|---|-----|----------------|

*The value was obtained by using dynamic light scattering measurements

$$* \text{Productivity} = \frac{\text{The content of initial Catechins}}{\text{Catechins in nanoparticles}} * 100\%$$

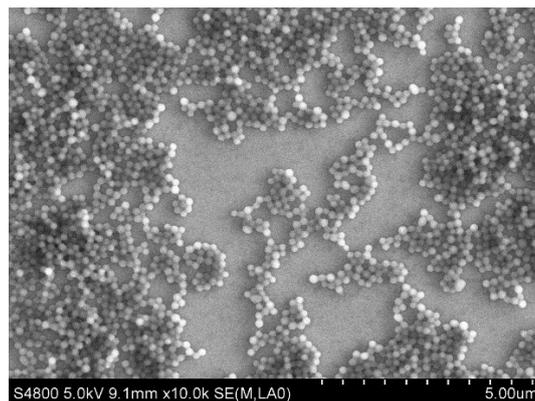


Figure S1. Low-magnification SEM image of np-1.

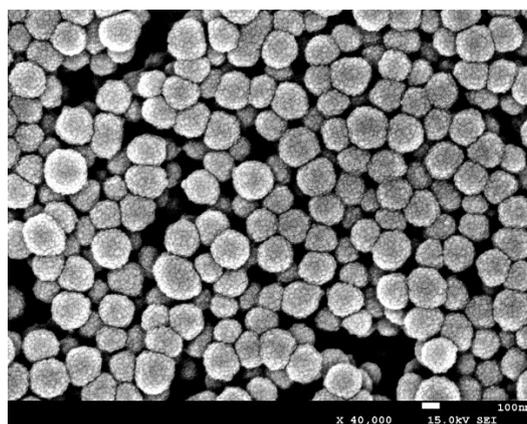


Figure S2. Low-magnification SEM image of np-2.

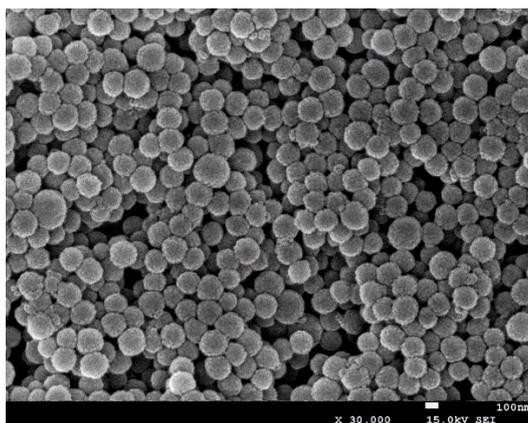


Figure S3. Low-magnification SEM image of np-3.

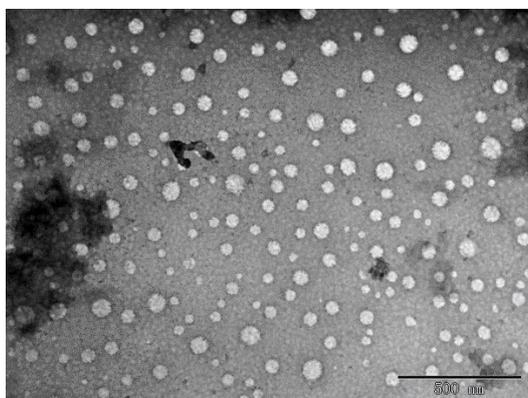


Figure S4. Low-magnification TEM image of np-4.

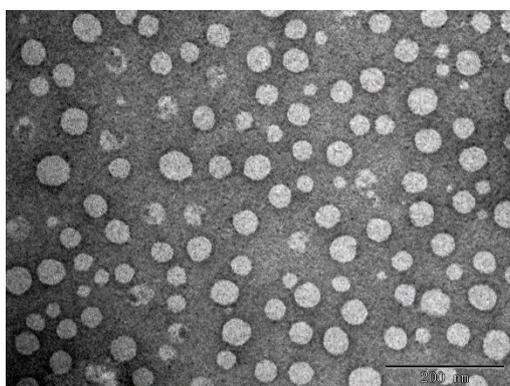


Figure S5. Low-magnification TEM image of np-5.

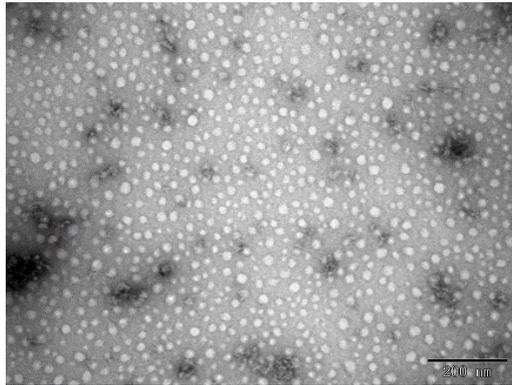


Figure S6. Low-magnification TEM image of np-6.

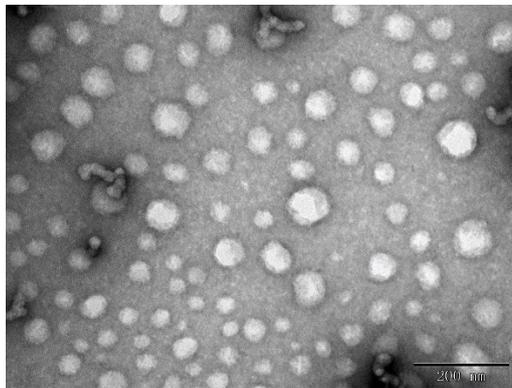


Figure S7. Low-magnification TEM image of np-7.

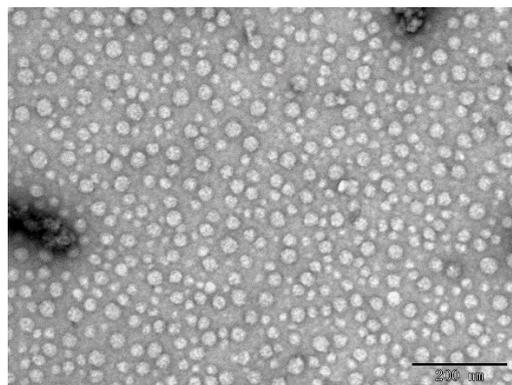


Figure S8. Low-magnification TEM image of np-8.

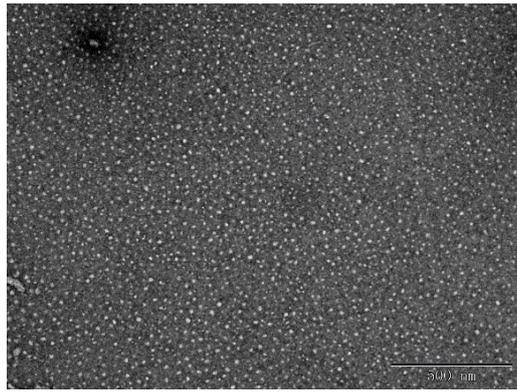


Figure S9. Low-magnification TEM image of np-9.

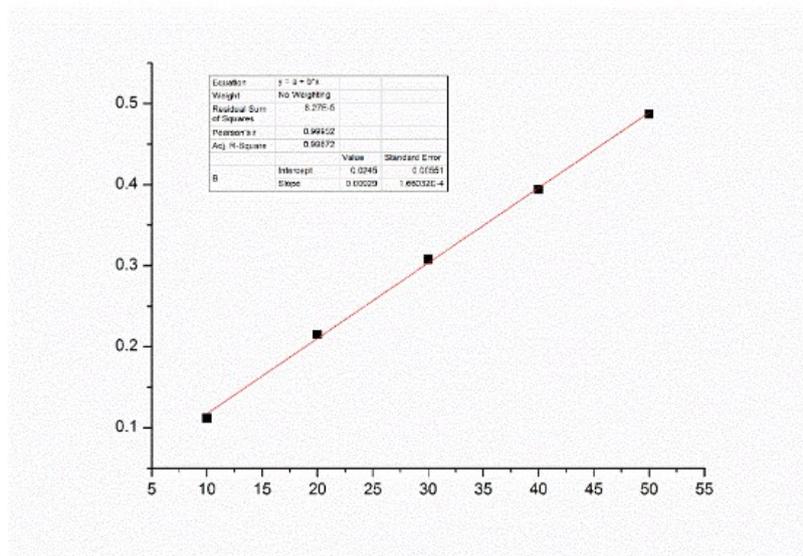


Figure S10. Calibration curve of catechins in water determined by UV-vis spectroscopy through Folin-Ciocalteu method.

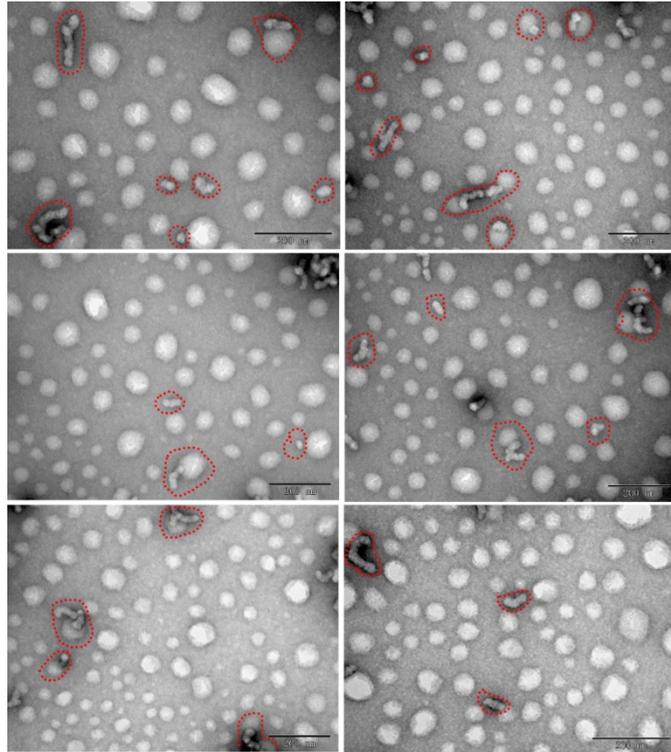


Figure S11. Typical worm-like nanoparticles in np-7 samples.

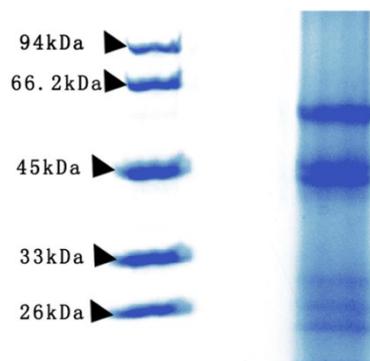


Figure S12. SDS-Page of keratin.

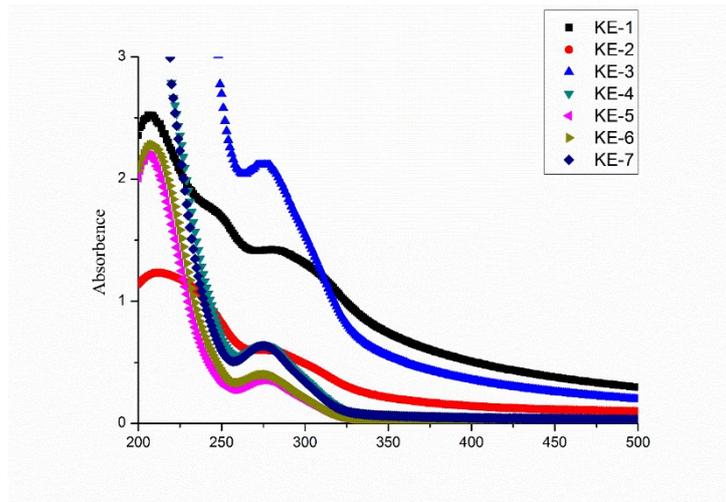


Figure S13. UV-vis spectra of np-1 to np-7.

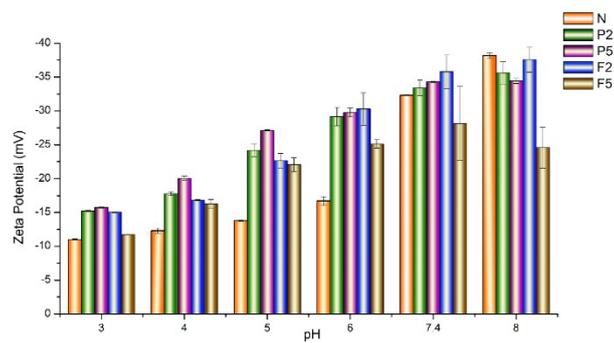


Figure S14. Zeta potentials of np-2, np-4, np-5, np-7 and np-8 measured at varying pH values of buffer solutions.

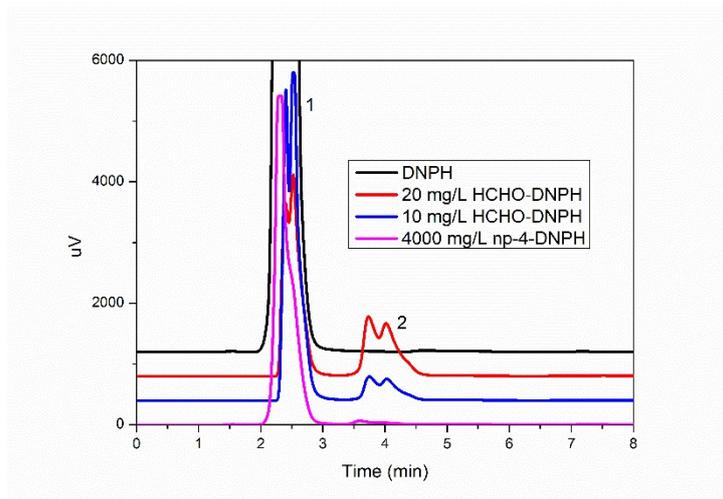


Figure S15. HPLC results of residual formaldehyde. 1) 2,4-DNPH, 2) HCHO-DNPH



Figure S16. Comparative photos of pure keratin and np-4 in ethyl alcohol. The immediate precipitation occurs for pure keratin whereas clear and transparent solution is observed for np-4.

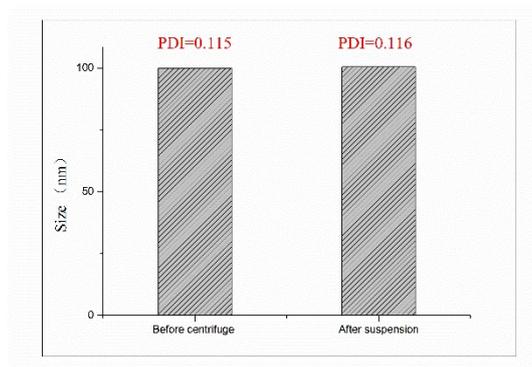


Figure S17. DLS data of nanoparticles before centrifugation (left) and re-dispersed in water after collected by salting-out method (right), showing the good polydispersity index.

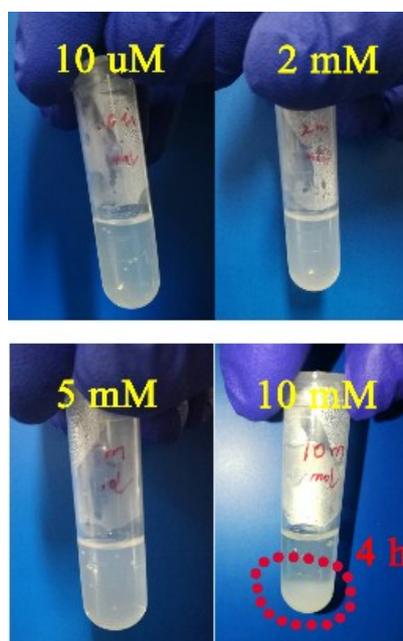


Figure S18. Nanoparticles of np-4 were incubated at 37 °C in different GSH concentrations of 10 μ M, 2 mM, 5 mM and 10 mM for 4 h. The sedimentation is observed only for nanoparticles incubated in 10 mM GSH, due to continuously swelling and subsequent breaking of nanoparticles into precipitated pieces.

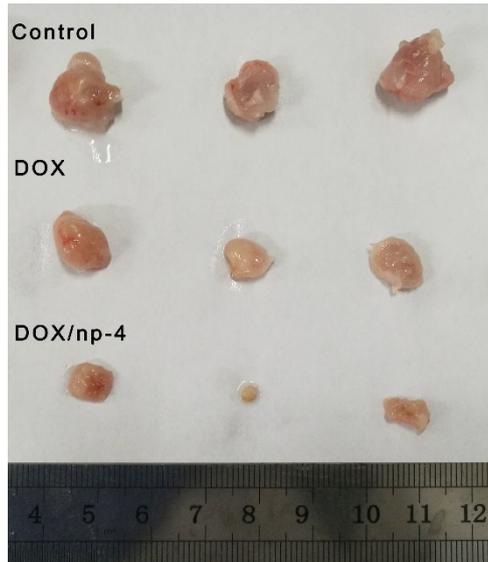


Figure S19. The photo of excised tumors from Bab/c mice bearing 4T1 tumor after treatment for 18 days.

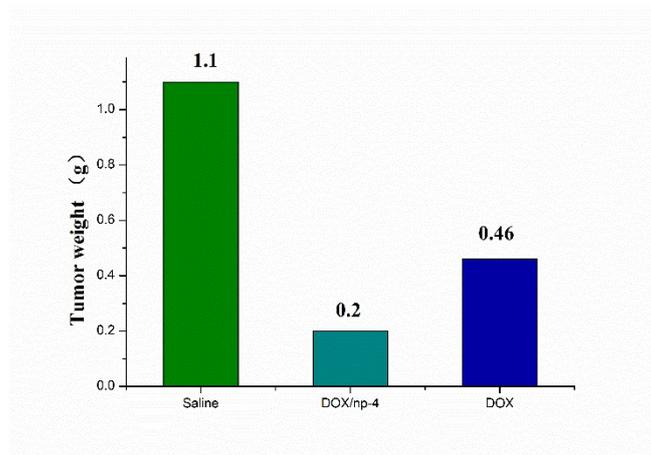


Figure S20. Tumor weight of excised tumors from Bab/c mice bearing 4T1 tumor after treatment for 18 days.