Supporting Information for:

Biological stimuli responsive drug carriers based on keratin for triggerable drug delivery†

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Extraction of keratin from wool and amino acid analysis [1-3]

The wool was washed in mild detergent and acetone to remove surface oil and then dried and cut into short pieces. The pretreated wool (40 g) was mixed with 8M urea (800 mL), sodium dodecyl sulfate (SDS, 1 g), and 2-mercaptoethanol (30 mL). The mixture was stirred at 65 °C for 24 h and then centrifuged at 12,000 rpm for 5 minutes and filtered. The obtained filtrate was dialyzed against deionized water using a dialysis bag with the cutoff molecular weight of 7 kDa and the out water was replaced every 12 h.

The amino acid composition of keratin was determined after hydrolysis with 6 N HCl at 110 °C for 24 h in a nitrogen atmosphere and the subsequent analysis was performed on L8800 (HITACHI of Japan). The quantitative amino acid composition was determined by calibration with the external standard Amino Acid Standard H from Pierce and there were 17 amino acids identified in keratin extracted from wool. Aspartic and Glutamic acids are resulted from hydrolyzing of asparagine and glutamine side chains.[4] Glutamic acid and proline were the most three abundant amino acids and the

cystine in the keratin is the sulfur-containing amino acid present in keratin. In particular, cystine is contributed to the disulfide bonds in the wool and can afford cysteine after reduction.[1]

Table S1. Amino acid composition of keratin extracted from wool.

Amino acid	Keratin (wt%)
Aspartic acid	5.88
Threonine	5.07
Serine	8.99
Glutamic acid	8.74
Proline	9.51
Glycine	3.03
Alanine	3.54
Cystine	5.07
Valine	4.33
Methionine	0.19
Isoleucine	2.73
Leucine	6.47
Tyrosine	2.98
Phenylalanine	2.04
Lysine	1.2
Histidine	Undetected
Arginine	7.49
Tryptophane	0.34

The molecular weight of the resultant keratin was estimated by SDS-PAGE analysis. In the gel image, three main bands at 14, 28, and 45 kDa are clearly seen in the gel image. The bands at 28 and 45 kDa represent the keratin with α-helical tertiary structure. With high sulfur content and lower molecular weight, γ-keratin can be seen at 14 kDa.[5]

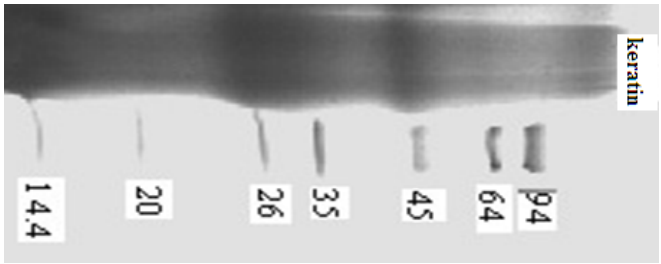

Figure S1. The elctrophoretical separation patterns of the keratin by SDS-PAGE.

The FTIR spectra were conducted on a Bruker-Equinox 55 FT-TR spectrometer at a resolution of 4 cm^{-1}. Each sample for infrared analysis was prepared using the KBr pellet technique. [3, 5] Infrared spectra of keratin shows characteristic bands assigned to the peptide bonds of protein.[3] The peptide bands are Amide A (3280 cm^{-1}), Amide I (1700-1600 cm^{-1}), Amide II (1520 cm^{-1}) and Amide III (1220-1300 cm^{-1}). The bands are consistent to that of peptide bonds of protein, which reveals that the keratin extracted from wool maintains the structure of protein as expected.

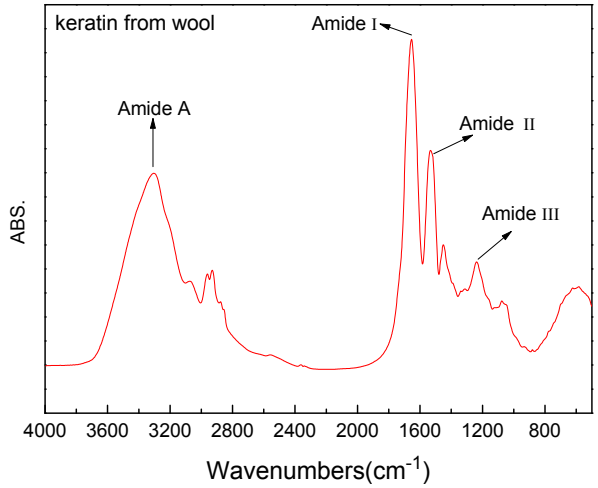

Figure S2. Fourier transform infrared (FT-IR) spectra of keratin**.**

The content of thiol groups was calculated from the sulfur content of keratin ($n_{\text{-SH}} = C_{\text{S·keratin}}/\text{Ar(S)}$). The sulfur content of keratin is 3.96%. Taking sulfur content in cystine and methionine (Amino acid analysis) away, the thiol groups in 1 g of keratin was 0.82 mmol g^{-1}. The keratin and PEG content of 1 g of graft copolymer ($C_{\text{k.keratin-}g\text{-PEG}}$, $C_{\text{PEG.keratin-}g\text{-PEG}}$) and DS were calculated by using the following expressions,

$$C_{\text{k.keratin-}g\text{-PEG}}\,(\%) = (C_{\text{S·keratin-}g\text{-PEG}} \times 1\text{g}) / C_{\text{S·keratin}} \times 100 \quad (1)$$

$$C_{\text{PEG.keratin-}g\text{-PEG}}\,(\%) = 1 - C_{\text{k.keratin-}g\text{-PEG}}\,(\%) \quad (2)$$

$$\text{DS} = n_{\text{PEG}} / n_{\text{-SH.keratin-}g\text{-PEG}} \quad (3)$$

where $C_{\text{S·keratin-}g\text{-PEG}}$ and $C_{\text{S·keratin}}$ are sulfur content of keratin-*g*-PEG and keratin. n_{PEG} and $n_{\text{-SH.keratin-}g\text{-PEG}}$ are moles of PEG chains of keratin-*g*-PEG and thiol groups of keratin composed of

keratin-*g*-PEG, respectively. The content of free thiol groups of keratin and graft copolymers were determined by Ellman's assay. In the method, 50 μL DTNB (10 mM, pH 7.0) was added and the absorption at 412 nm was recorded in 15 minutes on a Shimadzu UV-1601PC spectrophotometer. Then, the absorption at 412 nm of keratin diluted in PBS (pH 8.0, 100 mM) was read to estimate the thiol groups of keratin and copolymers.

Table S2. Results of elemental analysis of keratin and keratin-*g*-PEG copolymers

Samples	S content (%)		S content average (%)	DS^a	DS^b	Keratin content (%)[a]
1 keratin-*g*-PEG	1.91	1.93	1.92	0.25	0.15	48
2 keratin-*g*-PEG	1.53	1.41	1.47	0.41	0.45	37
3 keratin-*g*-PEG	0.58	0.54	0.56	0.97	0.87	14
4 keratin	4.03	3.90	3.96	0	-	100

[a] Estimated by elemental analysis.

[b] Estimated by Ellman's assay.

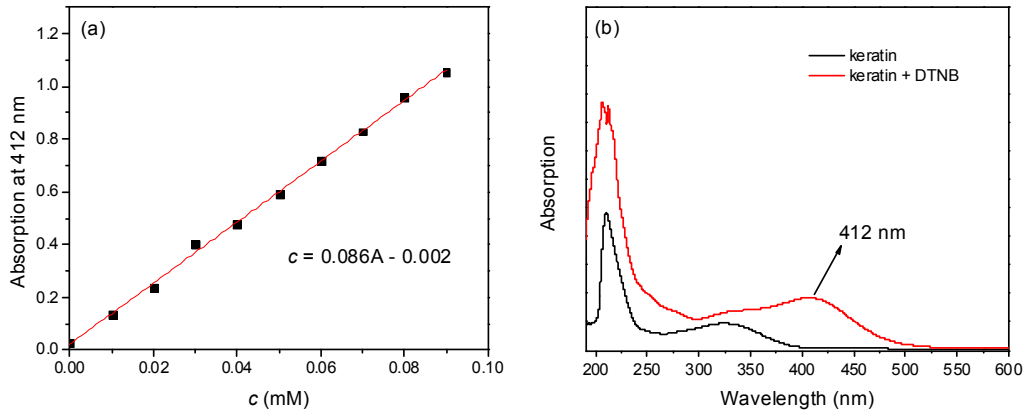

Figure S3. Estimation of the concentration of thiol groups. (a) absorption at 412 nm as a function of thiol group concentration, and (b) the UV-vis spectra of keratin solutions with and without DTNB.

CMC of keratin-*g*-PEG graft copolymer was estimated by the fluorescence probe technique. Pyrene was used as the probe and the concentration of pyrene was kept at 5.0×10^{-7} mol L^{-1} in all the solutions with different keratin-*g*-PEG concentrations (1.0×10^{-6} – 1.0 mg mL^{-1}). Then emission fluorescence spectra (excited at 393 nm) of the solutions were recorded. The intensity ratio of the peak at 338 nm to 336 nm (I_{338}/I_{336}) in the excitation spectra, which relates to polarity of the microenvironment around pyrene, was plotted as a function of copolymer concentrations. The turn point was used as the CMC of the keratin-*g*-PEG graft copolymers. The CMC value of

keratin$_{0.25}$-*g*-PEG was determined (6 μg mL^{-1}, Figure S4b). This means the keratin$_{0.25}$-*g*-PEG copolymer will aggregate into nanoparticles when the concentration is above 6 μg mL^{-1}. The CMC of the keratin-*g*-PEG copolymers increases with the increase in the graft density because of the increased hydrophilicity of the graft copolymer.

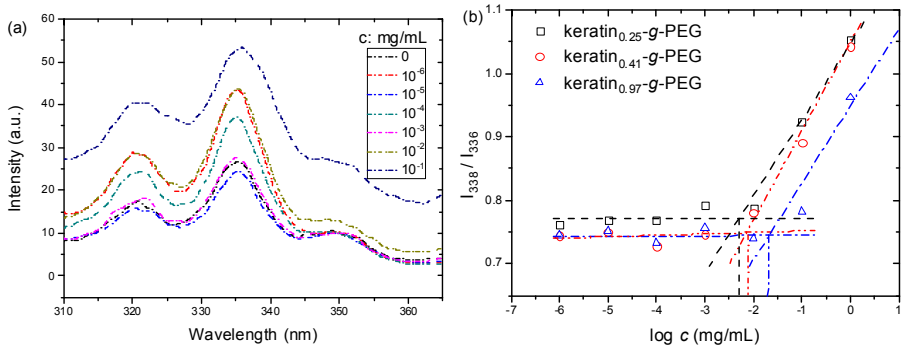

Figure S4. CMC of keratin-*g*-PEG graft copolymer by pyrene.

The fluorescence intensity of DOX released from the nanoparticles emission at 594 nm in response to varied GSH concentration in PBS was monitored for 2 days. The slit bandwidth of the measurements is (5, 10) for (a) and (b), and (10, 10) for (c), respectively. For the nanoparticles prepared from keratin$_{0.25}$-g-PEG copolymer, the maximum fluorescence intensity at 594 nm was reached after 12 h at the GSH concentration of 10 mM in pH 7.4 buffer solution (Figure S4a). For the cross-linked nanoparticles prepared from graft copolymer keratin$_{0.41}$-*g*-PEG and keratin$_{0.97}$-*g*-PEG, the maximum fluorescence intensity was reached after 24 h and 36 h at the GSH concentration of 10 mM (Figure S5b and S5c), respectively. The intensity of DOX released under 10 μM GSH was much weaker than that at 10 mM GSH at the same time intervals. It can be concluded that the release rate is dependent on the GSH concentration in the PBS.

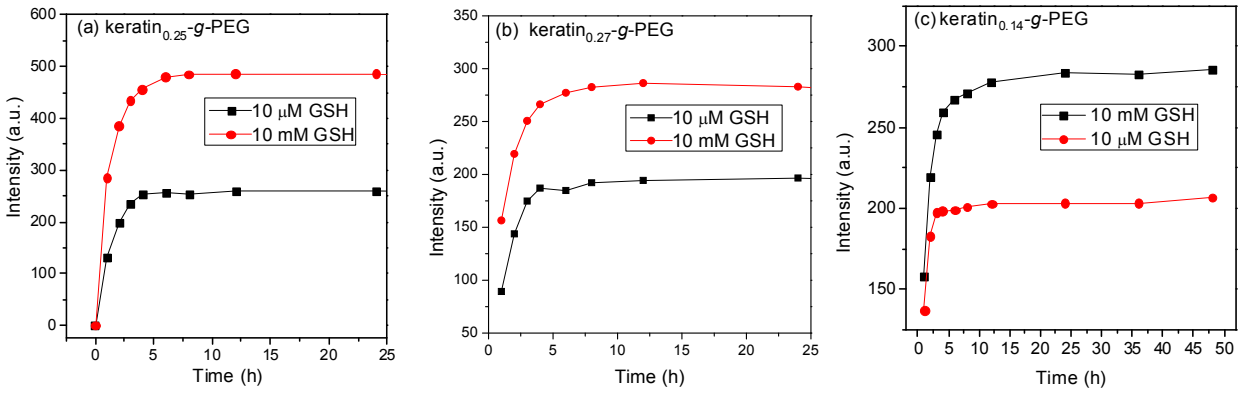

Figure S5. Fluorescence intensity of DOX released from the DOX-loaded nanoparticles.

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