Electronic Supplementary Information (ESI)

Hybrid cross-linked hydrogels based on fibrous protein/block copolymers and layered silicate nanoparticles: tunable thermosensitivity, biodegradability and mechanical durability

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Particle size characterization of Laponite

Dynamic light scattering (DLS) measurement was carried out on Zetasizer Nano ZS instrument (ZEN3600, Malvern, UK). Number particle size distribution of 0.5% w/v LP dispersion is shown in Fig. S1. The low concentration dispersion was used to avoid flocculation and possible particle aggregation in higher concentration dispersions. The size distribution vs. the number of particles obtained from DLS exhibits one narrow band indicating the presence of a majority population ranging from 20 to 40 nm with mean particle size of 30 nm (PDI=0.3). This narrow distribution confirms the homogeneous size distribution of LP nanoparticles which is in consistent with the known size of the disk-like nanoparticle.[1, 2] Based on literatures, Laponite RD clay consists of relatively monodisperse and well-defined platelets with an average diameter of 25–30 nm.[3-5]

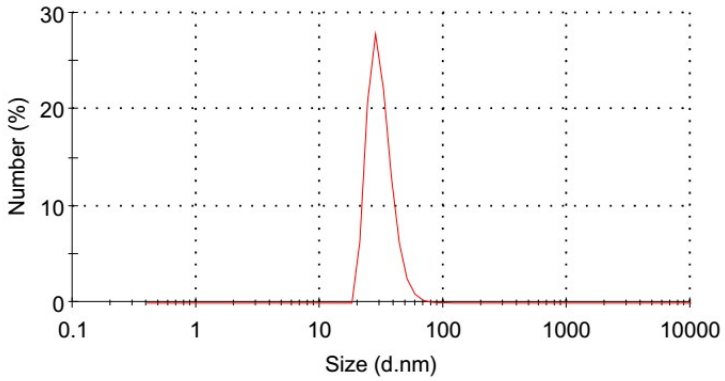


Fig. S1 DLS size distribution of LP nanoparticles.

Extraction of keratin from wool fibers

Keratin was extracted from New Zealand Merino wool (20 μm fineness) through the sulfitolysis reaction with slight modifications.[6, 7] First, fibers were washed in an aqueous solution containing a nonionic detergent (1 g/l) and sodium carbonate (1%) at liquor to fiber ratio of 40 ml/g for 30 min at 60°C, then rinsed thoroughly and dried. In order to remove the

fatty matters and grease, they were Soxhlet extracted with petroleum ether for approximately 12 h. The solvent was evaporated in ambient condition before rinsing the samples with distilled water. Cleaned defatted fibers were then chopped into short pieces and dried at room temperature (RT) before extraction procedure. The pretreated wool (10 g) was mixed with 200 ml aqueous solution containing 8M urea, 0.5M metabisulfite ($Na_2S_2O_5$), and 0.05M sodium dodecyl sulfate (SDS), adjusted to pH 6.5 with 5 N NaOH. The mixture was stirred at 65 °C for 24 h, filtered with a 0.45 μm pore-size stainless steel sieve, and dialyzed against distilled water using a cellulose tube (Sigma, USA, M_w cut-off =10 kDa) for 3 days at RT, changing distilled water three times a day. The keratin aqueous solution after dialysis was filtered again and finally lyophilized for 48 h by a vacuum freeze-dryer (Lyotrap/Plus, UK) at −40 °C in order to obtain pure keratin powder (extraction yield ~ 40%).

Characterization of regenerated keratin

The protein-content of the resultant keratin was determined by Hartree-Lowry method using bovine serum albumin as standard, which estimated the concentration of protein based on tyrosine amino acids present in the solution.[8, 9] Sulfhydryl (thiol) group concentration was also measured by using DTNB reagent, 5,5'-dithio-bis-(2-nitrobenzoic acid), in phosphate buffer (100 mM, pH=8) according to the colorimetric Ellman assay at 412 nm.[10] To study the chemical structure of keratin, Fourier transform infrared (FTIR) analysis was performed by FTIR spectrophotometer (ABB Bomem MB100, USA). The characteristic spectra were scanned in the wave number range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} using KBr pellets. In addition, the molecular weight was estimated by electrophoresis SDS-polyacrylamide gel (SDS-PAGE) according to the Laemmli method[11] using the Mini-PROTEAN 3 Cell system from Bio-Rad. The 12% polyacrylamide gel was processed at 100V in running buffer, Tris–glycine SDS. Proteins were stained with Coomassie brilliant

blue R (Sigma-Aldrich, USA) and analyzed based on a wide range molecular weight marker (Fermentas, USA, MW 10-170 kDa).

Results

For keratin extraction, the reducing agent, $Na_2S_2O_5$, was used to break down the disulfide bonds along with urea as a denaturing agent to cleave hydrogen bonds in protein. SDS not only accelerated the extraction rate by lowering the surface tension between the solid substrate (wool) and the reagents, but it also could act as a stabilizing agent for regenerated keratin.[12] The content of released protein and free thiol groups of keratin were determined 12.7 mg/ml and 0.82 mM, respectively. The FTIR spectra of extracted keratin in Fig. S2 shows characteristic absorption bands assigned mainly to the peptide bond (CONH) which represents the fundamental structural unit of the polypeptide chain of the protein. The broad absorption band region from 3600 to 3200 cm^{-1} can be attributed to the stretching vibration of N–H and O–H bonds. Peaks that fall in the 3000-2800 cm^{-1} range are related to C–H stretching modes of aliphatic groups. The vibrations in the peptide bonds originate bands known as amide I, II, III. The amide I band is connected mainly with the C=O stretching vibration and it occurs in the range of 1700–1600 cm^{-1}, while the amide II falls at 1535 cm^{-1} which is related to N–H bending and C–H stretching vibration. The amide III band at 1237 cm^{-1} results from in-phase combination of C–N stretching and N–H in-plane bending.[13, 14] The position and intensity variability of amide bands are attributed to the conformational changes in keratin.[15] On the basis of the literature data, the absorption at 1650 cm^{-1} indicates the presence of crystalline α-helix structures, while the band between 1610-1633 cm^{-1} and 1675-1695 cm^{-1} are typically found for β-sheet assembling and disordered conformation, respectively.[16] Therefore, the absorbing peaks in the amide region imply that the extracted keratin had dominant crystalline α-helix microstructure.

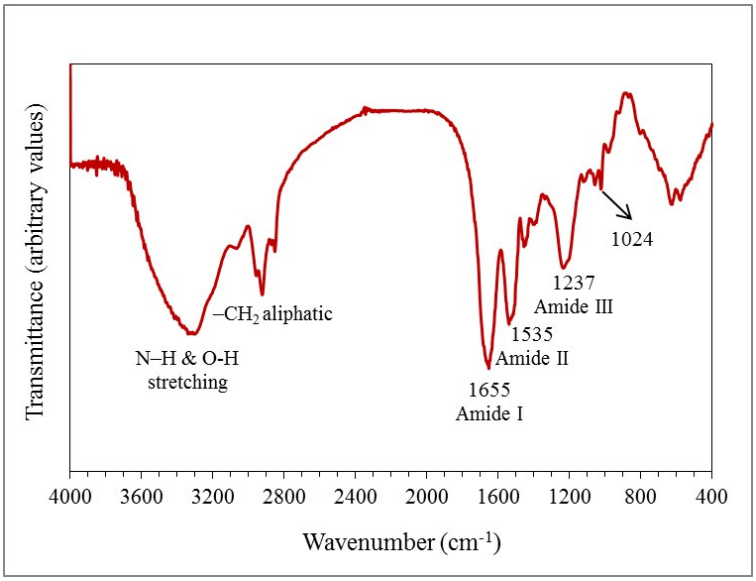
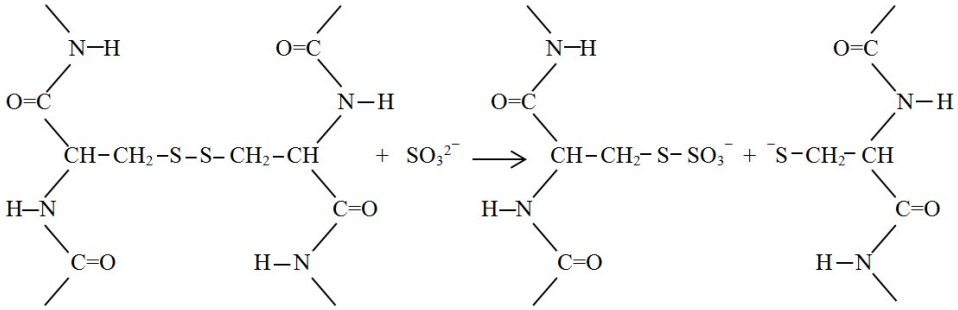


Fig. S2 The FTIR spectra of the extracted keratin from wool.

Besides, the band at 1024 cm^{-1} is associated with the symmetric S–O stretching vibrations of the Bunte salt residues as a result of the cleavage of disulfide linkages by the reducing agent.[17] During sulfitolysis, cystine disulfide bonds are broken by sulfites to give cysteine thiol and Bunte salt (S-sulfo group), according to Scheme S1.



Scheme S1. Reduction of disulfide bonds in keratin by sulfitolysis.

Fig. S3 represents the electrophoretic separation pattern of the extracted keratin. A formulation of high molecular weight standard between 10-170 kDa was applied to lane 1 for calibration. The pattern in lane 2 shows clear protein fractions between 40-60 kDa, related to the low-sulfur keratin from the intermediate filament proteins (IFPs) of fiber cortex with

typical α-helix secondary structure.[14] The hard α-keratins are further resolved into two subfamilies, consisting of Type I acidic microfibrillar component (40-50 kDa) and Type II neutral/basic (55-65 kDa) members. The smear of lower molecular weight fractions between 11-30 kDa is related to the high-sulfur protein of the amorphous matrix embedding the intermediate filaments.[18, 19] The pattern indicates that the extracted keratin is mainly composed of low-sulfur α-keratin having helical structure confirming the FTIR results.

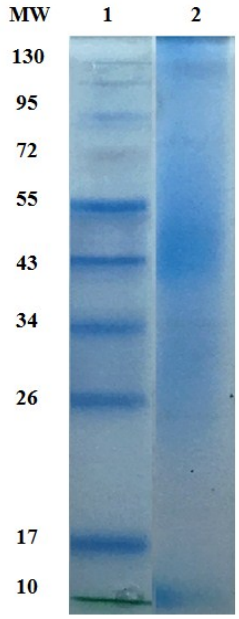


Fig. S3 The electrophoretic separation pattern of keratin by SDS-PAGE.

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