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Supporting Information

Cashmere-derived keratin for device manufacturing on the micro- and nanoscale

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Experimental details

Keratin extraction: Keratin is a family of proteins. Extraction of the proteins that form cashmere fibers yields not a single protein but several proteins, which falls under the name of keratins. For simplicity and clearness to non-technical experts, in the manuscripts we referred to this pool of proteins as keratin, considering it as a single entity. Keratin extraction was performed with a previously reported method [1], with some modifications. In brief, raw, white cashmere wool (30g) was rinse in distilled water (6 liters, 30°C) for 30 minutes (three water changes every 10 minutes), blotted and dried under vacuum for 6 hours. Lipid extraction was then performed with 100% acetone for 24 hours to remove remaining unbound surface lipids. The fibers were then washed (3x) with distilled water (6 liters, 30°C) for 3 hours (three water changes every 45 min) and air-dried. Delipided cashmere fibers were then cut into short fibers, 3 mm long. A mixture of 7M urea (500 ml), 2-mercaptoethanol (50 ml) and 0.5M thiolurea (50 ml), was used to solubilized 30 g of cashmere at 50°C for 72h. The solution was then filtered through a stainless steel sieve (#200) and dialyzed in dialysis tubes (3,500 MW cut-off) against distilled water (8 liters) for 72 hours (changed every 6 hours). The so obtained keratin solution was then centrifuged twice (5°C, 9000 rpm, 20 min per cycle) to remove insoluble particles, resulting in a clear protein solution (0.7±0.3 wt%), which was then concentrated to 2 wt% through a centrifugal evaporator. The total extraction yield was 47±5%.

Film Preparation and slow drying process: Keratin films were fabricated by solvent casting keratin solution on PDMS molds. Slow drying process was achieved by controlling the relative humidity environment (RH=30-95%) during keratin solution solvent casting by using a custom made humidity chamber. Diffractive PDMS mold and PDMS-made multi-lens arrays were fabricated using optical diffraction gratings (Edmund Optics, 300-1200 lines/mm)

or optical cards (Digital Optics Corp., Tessera Technologies Inc.) as masters. After drying, films were let acclimated to RH=30% and then carefully lifted from the mold. Film thickness was controlled by varying either keratin concentration or solution volume used during casting.

Inverse opal fabrication: PMMA nanospheres (∅=250 nm) were used to fabricate an opal template (1% concentration dispersed in water, Phosphorex). The PMMA solution was deposited onto a silicon wafer, which was then heated on a hotplate at 90 °C to generate the PMMA opal by self-assembly induced by water evaporation. The keratin solution was then added to the PMMA opal and filled the air voids by capillary infiltration. The solution was set to dry in a film at room temperature and RH=95%. The so formed keratin film was soaked in acetone for 24 h to allow for detachment from the silicon wafer and removal of the PMMA nanospheres.

Measurement of regenerated keratin molecular weight and purity: Cashmere-derived keratin was diluted 1:1 with a solution of 2x Laemmli sample buffer, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and 0.125 M Tris–HCl. Keratins were then run in a vertical slab gel electrophoretic system at 200 V, 80 mA, and 25W. A solution of 0.1% Coomassie brilliant blue R-250 (Sigma),10% acetic acid, and 40% methanol was then used to stain the keratin in the gel for 1 h. Excess of staining was then removed by rinsing the gel in deionized water overnight.

Physical characterization: Scanning electron microscopy (SEM) was used to investigate keratin film morphology and to determine surface patterning. Casted films were mounted on carbon tape and sputter coated with platinum-palladium. The films were then imaged using a scanning electron microscope (Supra55VP , Zeiss). Atomic force microscopy (AFM) was used to investigate surface morphology and to determine surface roughness of non-patterned and patterned keratin films. Micrographs of keratin films were acquired with a Digital Instrument Dimension 3100 (Veeco Instruments, Inc.) in tapping mode. Keratin film spectrum

in the visible wavelengths was measured with a USB2000 Miniature Fiber Optic Spectrometer**.** A Metricon waveguide instrument was used to evaluate refractive index of keratin films. The measured indices of refraction and film thicknesses are evaluated at a wavelength of $\lambda = 633$ nm, as previously reported for silk fibroin.

Spectroscopical characterization: Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy of keratin films was performed with a Jasco FT/IR-6200 Spectrometer, equipped with a multiple reflection, horizontal MIRacle™ attachment (Ge crystal, from Pike Tech., Madison, WI). Each collected spectrum was obtained as an average of 128 scans with a wavenumber range of 4000-650 cm^{-1} and a nominal resolution of 4 cm^{-1}. To analyze keratin conformational changes as a function of slow drying processing, Amide I and Amide III peak absorptions were analyzed as previously reported [2, 3]. Micro-Raman spectroscopy was performed with a Jasco NRS-3000 spectrometer in the 2000-400 cm^{-1} range using a 733 laser and a 100x objective. Each spectra was collected as an average of 20 scans (10s per scan) with a resolution of 1 cm^{-1}. Cosmic rays removal, measurement of FWHM and of I_{850}/I_{830} ratio were performed with Jasco Spectra Analysis software. Ellman**'**s reagent was used to determine the content of free thiol groups of cysteine residues in the cashmere-derived regenerated keratin solution and in assembled films [4].

References

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Table S1. Reduced cysteine content (measured as free thiol group through Ellman's reagent assay) in cashmere-derived regenerated keratin solution, in keratin films assembled at increasing relative humidity values, and in keratin films after acetone post-treatment

Keratin material [slow-drying relative humidity, %]	Free thiol group concentration [mM]
Keratin solution	0.976±0.231
Film [RH=50%]	0.061±0.018
Film [RH=75%]	0.143±0.041
Film [RH=95%]	0.579±0.074
Film [RH=95%] – Acetone post-treatment	0.302±0.027

Figure S1

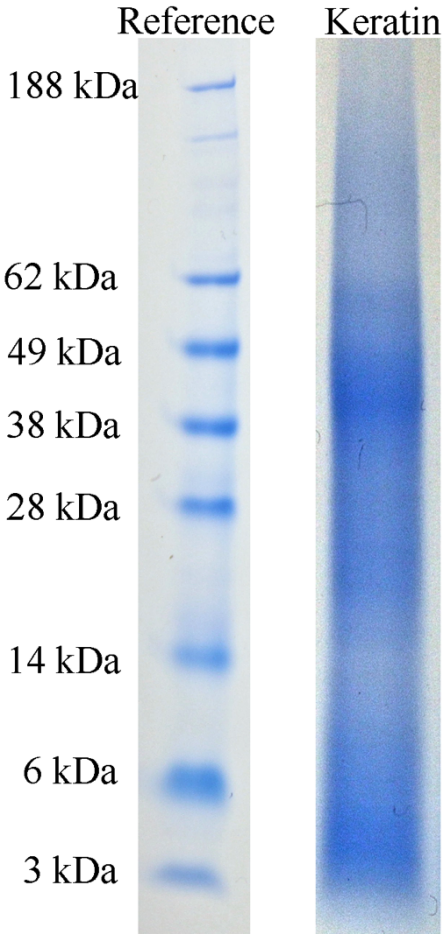

Figure S1. SDS-PAGE of standard protein molecular weight markers (left lane) and cashmere-extracted keratin (right lane).

Figure S2

Figure S2. Photograph of projected patterns obtained from propagation of a green light laser source through keratin-made 2D diffractive phase masks. The images are taken at a distance of 10 cm from the keratin optical element. (Master from Digital Optics Inc., Tessera Corporation).