A Bioinspired and Hierarchically Structured Shape-Memory Material

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By *Luca Cera, Grant M. Gonzalez, Qihan Liu, , Suji Choi, Christophe O. Chantre, Juncheol Lee, Rudy Gabardi, Myung C. Choi, Kwanwoo Shin, and Kevin Kit Parker**

[*] Prof. Kevin Kit Parker, Luca Cera, Qihan Liu, Grant M. Gonzalez, Christophe O. Chantre, Suji Choi, Rudy Gabardi
Disease Biophysics Group
John A. Paulson School of Engineering and Applied Sciences, Harvard University
Cambridge, MA, USA

Juncheol Lee, Prof. Myung Chul Choi.
Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea.

Prof. Kwanwoo Shin.
Department of Chemistry and Institute of Biological Interfaces, Sogang University, Seoul, 121-742, Republic of Korea.

Corresponding author:
[*]Prof. Kevin Kit Parker
29 Oxford St (Rm 321)
Cambridge, MA 02138
Phone: (617) 495-2850
Fax: (617) 495-9837
E-mail:

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Abstract

Shape memory polymeric materials lack long-range molecular order enabling more controlled and efficient actuation mechanisms. Here, we develop a hierarchical structured keratin-based system that has long-range molecular order and shape memory properties in response to hydration. We explore the metastable reconfiguration of keratin secondary structure – α-helix-to-β-sheet transition – as an actuation mechanism to design a high-strength shape memory material that is biocompatible and processable through fiber spinning and 3D printing. We extract keratin protofibrils from animal hair and subject them to shear stress to induce their self-organization into a nematic phase, which recapitulates the native hierarchical organization of the protein. This self-assembly process can be tuned to create materials with desired anisotropic structuring and responsiveness. Our combination of bottom-up assembly and top-down manufacturing allows for the scalable fabrication of strong and hierarchically structured shape memory fibers and 3D printed scaffolds with potential applications in bioengineering and smart textiles.

The growing demand of shape memory (SM) devices in the fields of civil engineering,[1] aerospace,[2] wearable technology,[3] and medical devices,[4,5] has galvanized research beyond the conventional metal alloy architype and towards the design of more tailorable polymeric SM materials[6] with improved bio-compatibility and bio-degradability properties.[7] Despite the wide variety of reported systems, controlling the spatial organization of the actuation mechanisms at the molecular level through all spatial scales remains a challenge. Achieving a long-range order of the molecular actuators would lead to a much tighter control over mechanical transformation and higher-level behavioral complexity.[8]

While traditionally associated with synthetic materials, shape memory features have also been observed in biological substrates as a result of the structural metastability of protein secondary structures.[9] In this regard, the keratin α-helices arranged in a coiled-coil architecture are known to undergo continuous structural transition into metastable β-sheets when load is applied along their longitudinal axis.[10,11] Depending on the α-keratin species, this mechanism can be either irreversible or reversible, with the latter resembling the martensitic SM mechanism of metal alloys.[12] In biological tissues such as sea snail egg capsules[13] or animal skin,[14] this mechanical transformation has been selected by nature to guarantee protection and enable physiological functioning in response to external stress. This SM mechanism was also recently observed in certain hair species.[15]

In this work, the reversable keratin α-helix-to-β-sheet transition is inspired by nature and hypothesized as the main mechanism for the design of a highly processable and nanostructured SM material, that uses hydration as trigger and is inherently biocompatible and biodegradable. Our design approach aims at the recapitulation of the α-keratin hierarchical and anisotropic organization present in animal hair, which allows for the engineering of strong and stiff water triggered SM (WTSM) devices with tensile strength and Young's modulus orders of magnitude greater than conventional systems.[16,17,18,19,20,21] This is achieved by starting from a non-destructive extraction of keratin protofibrils from animal hair. Keratin protofibrils were found

to self-organize into a nematic phase under shear stress, and their anisotropic alignment was further tuned by the charge screening effect to achieve protofibrils alignment during the extrusion process. In addition to the scalable production of continuous shape memory fibers, the material processability extended its use to 3D printing to fabricate shape memory architectures capable of complex geometrical transformations. Through the design of our keratin-based shape memory material, we introduced the use of protein structural metastability as an actuation mechanism for the engineering of nanostructured smart materials having long-range molecular order.

Keratin Extraction and Self-organization

In animal hairs, the strain-induced α-helix-to-β-sheet transition is feasible due to the paired configuration of α-helices into a coiled-coil architecture (Fig. 1a).[22] Coiled-coils are hierarchically self-assembled into an anisotropic fibrillar structure going from protofibrils up to macrofibrils, enabling continuity of the mechanical transformation through all spatial scales. Here, fibrillar keratin was extracted from Angora wool using lithium bromide (LiBr), a salt which is able to induce a reversible solid-to-liquid phase transition of crystalline keratin in water.[23] Breaking down the dense disulfide network of the hair matrix component is another requirement to set the fibrous keratin free from the hair structure. This was accomplished by using 4-dithiothereitol (DTT), capable of cleaving the disulfide bond by yielding two sulfhydryl moieties.[24] This reaction is reversable under oxidative conditions, thus allowing for the reconstitution of the native disulfide bridges during material fabrication. Keratin was then successfully extracted by treating ground wool with a water solution of LiBr and DTT at high temperature (Fig. 1b). At room temperature, keratin was finally isolated via liquid-liquid phase separation, process which was enhanced upon increase of ionic strength and further storage at low temperature. This last step of the protocol allowed for the formulation of a highly concentrated keratin solution (Supplementary Fig. 1), showing a shelf life of weeks when stored

in absence of oxygen. Its relatively high protein content makes the extracted solution an ideal substrate for a variety of material fabrication processes. The preservation of the coiled-coil architecture of the keratin α-helices was initially confirmed by Raman spectroscopy and circular dichroism (Supplementary Fig. 2 and Supplementary Fig. 3). This was further supported by SDS-PAGE analysis, which showed the typical bands of the coiled-coils dimer and also tetramer, most likely of heterodimeric nature as reported for Angora wool, with very few protein degradation products (Supplementary Fig. 4).

Evidence of the keratin hierarchical structure up to the protofibrillar level was finally supported by cryogenic transmission electron microscopy (cryo-TEM). Figure 1c shows the presence of bundles up the micron range in length and with a varying width maximum value of approximately 10 nm matching the intermediate filaments (IF) structural features.[22] Magnification of the cryo-TEM micrograph also elucidates the internal hierarchical structure of the IF composed of packed protofibrils with a consistent width of ~ 3 nm.[22]

The recapitulation of the hierarchical architecture of keratin, enabling the long-range order of the α-helix actuation units, requires the imposition of anisotropic alignment of the protofibrils during the fabrication process. In this regard, keratin protofibrils were found to self-organize into a nematic crystal phase when subjected to shear stress and spatial constraint. This was initially deduced from the anisotropic nature of the synchrotron X-ray scattering pattern obtained from a sample of the keratin solution (401.7 mg/mL) prepared in a quartz capillary (Fig. 1d).[25] During the analysis, the capillary was placed perpendicular to the X-ray beam and with its longitudinal axis parallel to the meridian axis of the detector. The equatorial nature of the scattering pattern suggests the keratin domains to be preferentially oriented parallel to the capillary axis (Fig. 1d, inset). The average distance among the keratin domains is associated to the lattice size parameter d, which is obtained from the maximal intensity of the scattering vector modulus q as defined by $d = 2\pi/q$.[25] The nematic ordering of the keratin protofilament is reasoned as the result of the shear stress generated at the capillary wall during sample

preparation and further stabilized by the space constraint.[25] In this scenario, enhancement in stiffness and self-assembly of the keratin protofibrils is expected to lead to a higher degree of order of the nematic phase. Thus, tighter control over the self-organization of the keratin liquid crystal phase was achieved by promoting protein-protein interactions via the charge screening effect.[26] Due to the presence of lithium cations absorbed on the protein surface, keratin is expected to have a net positive charge.[27] The phosphate anion is known to have a high screening effect towards positively charged surfaces[26] and therefore used for this purpose (Fig. 1e). As shown in Fig. 1f, the addition of NaH_2PO_4 indeed caused tightening of the keratin nematic phase packing, indicated by a peak shift towards a higher value of q. Upon addition of the kosmotropic salt, narrowing of the equatorial scattering pattern and consequent sharpening of the scattering peak were also observed, thus indicating increase of the keratin domain alignment along the capillary axis (Fig. 1g).

Tuning the keratin nematic phase organization via protein charge screening dramatically affects the rheological properties of the protein solution. Upon increase of NaH_2PO_4, aggregation of the protofibrils causes enhancement of the protein solution viscosity at low shear rates (Fig. 1h).[28] This finding is in agreement with the SAXS data showing a tighter packing of the keratin filaments upon addition of the kosmotropic salt. However, upon increase of shear rate, the alignment of the keratin protofibrils causes a sudden decrease in viscosity, thus conferring a pronounced shear thinning behavior to the protein solution. The presence of steeper slopes upon increase of NaH_2PO_4 further supports higher degree of alignment of the keratin protofibrils, which is induced by the stiffening of crystalized proteins. With a NaH_2PO_4 concentration of 40 mM and protofilaments concentration of (401.7 mg/mL), the keratin dope shows viscoelastic properties suitable for further processing, as fibers could be directly formed by simply pulling the protein with tweezers (Fig. 1i). Upon decrease of NaH_2PO_4 concentration, the keratin solution loses its viscoelastic properties, and fails to form fibers directly from the solution (Supplementary Fig. 5).

150

151 **Hierachical Structuring and Anisotropy in Keratin Fibers**

152 The alignment of the keratin α-helices along the fiber axis is a design criterion to ensure high
153 strength and high fixity yields to the fibers. When the α-helix axes are parallel to the pulling
154 vector, the maximum uncoiling of α-helices can be obtained, thus enabling a greater material
155 strain to failure due to plastic deformation and reorganization. To this point, the ability of the
156 keratin protofibrils to self-organize into a nematic phase is ideal to fabricate hierarchically
157 structured SM materials through extrusion processes. Thus, anisotropic keratin fibers could be
158 spun using a traditional wet-spinning platform. A water solution of NaH_2PO_4 is used as
159 antisolvent, allowing for both the outer diffusion of LiBr from the extruded keratin dope and
160 further self-assembly of the protein via the charge screening effect (Fig. 2a and Supplementary
161 Fig. 6). The restoration of the disulfide covalent network was enabled by the oxidative activity
162 of hydrogen peroxide (H_2O_2) on the cysteine thiol group.[29] The high protein concentration in
163 the dope confers robustness to the fiber during the coagulation process which allowed for a
164 flexible and reliable spinning process. Continuous and homogeneous fibers could be produced
165 (Fig. 2b), and high drawing rates also applied, thus allowing fiber diameters of 10 μm
166 (Supplementary Fig. 7).

167 The nematic phase organization of the keratin protofibrils leads to a fibrillation process
168 generating hierarchically structured and anisotropic fibers. Scanning electron microscopy
169 shows how a single fiber is composed of continuous fibrils which are at least a few tens of
170 micrometers long (Fig. 2b). The cross-sectional area confirms the fibrils to be in the 50 nm
171 range in diameter and constitute the core of the fiber (Fig. 2d). Polarized optical microscopy
172 supports the anisotropic nature of the fiber core by showing its birefringence behavior with a
173 maximum of the transmitted light intensity at 45° (Fig. 2e). The orientation of the α-helices
174 along the fiber axis was confirmed by polarized Raman, which shows highest intensities for the

amide I signal (C=O stretching mode) when the fiber is oriented parallel to the laser, for both the cross and non-cross combinations of the laser and the analyzer (Fig. 2f).[30]

Insights into the coiled-coils structure and anisotropic organization were obtained through wide-angle X-ray scattering analysis (WAXS). The 2D scattering profile shows the characteristic equatorial reflection at 9.65 Å, corresponding to the spacing between the axis of adjacent α-helices (Fig. 2g).[31] The one-dimensional analysis along the meridian axis shows the presence of the characteristic meridian and off-meridian reflections belonging to the α-helix pitch projection, and detected at 5.15 Å (shoulder) and 5.05 Å (maximum), respectively (Fig. 2h and Supplementary Fig. 8).[32] Compared to the Angora wool fiber, these reflections are however broader and have a different reciprocal intensity ratio, suggesting the α-helices to be less crystalline than the natural analogue (Supplementary Fig. 8). Furthermore, the scattering arc in the 5 Å equatorial region indicates that part of the α-helices is more randomly oriented (Fig. 2h). In addition, the fact that the maximum is shifted towards higher q values suggests also the presence of uncoiled peptide chains, which are oriented parallel to the fiber axis and most likely arranged into a β-sheet conformation (Fig. 2i).[31] The uncoiling of peptide chains is hypothesized to be caused by the tensile stress generated during fiber spinning, which leads to a partial denaturation of the coiled-coil anisotropic architecture.

Hydration-Responsive Shape Memory Fibers

The SM effect of the designed keratin fibers relies on the reversable uncoiling of the α-helix and formation of metastable β-sheets when uniaxial strain is applied (Fig. 3a) – a concept which was pioneered by Miserez et al.[13] Tensile tests carried out on single keratin fibers confirm this mechanism by showing an initial elastic behavior (Young's modulus 4.18 ± 0.10 GPa) up to ~ 5% strain (gray region), followed by a yielding region (blue region) characterized by a constant yield stress (96.1 ± 3.1 MPa), which corresponds to the α-helix unfolding process (Fig. 3b). As

the strain further increases, the uncoiled and elongated keratin peptide chains are stabilized in their stretched geometry by assembling into β-sheets (Fig. 3c). This β-sheet forming region is characterized by a strain hardening at ~ 50% of strain, as the applied load is not only dissipated by the disruption of the coiled coils, but also carried by the stretching of the β-sheets (cyan region). As load is removed at a 100 % (tensile strength 137.18 ± 1.03 MPa), the fiber shows a plastic strain (~ 85 %) in agreement with the entrapment of the keratin unfolded chains into the new metastable β-sheets. Overall, the mechanical properties of the designed keratin fibers match the ones of native wool hair.[33] The rearrangement of the protein secondary structure during this strain-induced α-helix-to-β-sheet transition was monitored by tracking the shift of the amide I Raman signal, known to have two distinct scattering bands, at 1652 cm^{-1} for the α-helix and at 1671 cm^{-1} for the β-sheet.[34] As shown in Figure 3d, a small fraction of β-sheets is already present in the unstrained fiber and in agreement with the WAXS data already provided. At 100% of strain, a blue shift of the amide I band is observed and integration of the deconvoluted peaks clearly suggests the increase of the β-sheets component upon conversion of the coiled-coils α-helices. The strain-induced α-helix-to-β-sheet transition was additionally supported by WAXS analysis, which shows the formation of the characteristic 4.65 Å equatorial reflection corresponding to the lateral distances between the peptide chains in a beta-sheet secondary structure (Fig. 3e).[31] Uncoiling of the α-helices is indicated by the decrease in intensity of the meridian reflections on the 2D pattern, and by the 5.05 Å and 5.15 Å maxima shifts towards higher values of q on the one-dimensional meridian scattering profile (Supplementary Fig. 9). The fact that the equatorial signal in the 9 Å region is almost unshifted, suggests that the β-sheet structures preserve the same spacing of the α-helices and therefore originate from the coiled-coil architectures. The change in the WAXS 2D pattern is in agreement with the α-helix-to-β-sheet transition mechanism occurring in natural fibers.

In the stretched fiber, β-sheets are kinetically stable due to the presence of the hydrogen bond network hindering their reconversion into the more thermodynamically stable α-helices. Leveraging this property, a shape memory cycle can thus be elaborated where the hydrogen bonding network functions as a locking mechanism ensuring the fixity of the deformed shape. Water is here used as a stimulus to both facilitate fiber deformation and recovery to the original shape (Fig. 3f). This concept was tested for a bundle of keratin fibers of same diameter size (Fig. 3g and Supplementary Video 1). The fiber bundle was hydrated in deionized water for a few seconds (state A), manually stretched in the air while still in a wet state (state B) and then kept under load at room temperature for 10 minutes to let the fiber dry (state C). When weights were removed to allow the fiber bundle to relax, no visible change in length between the stretched and relaxed form was noticeable to the naked eye (state D). The ability of the bundle to recover its original length was then confirmed by applying nebulized deionized water, which triggered shrinking of the fibers back to their original length within a few seconds (state A'). When compared to the experiment conducted in dry state (Fig. 3b), tensile tests clearly showed the role of water in facilitating the protein structure rearrangement. This is indicated by an overall decrease in tensile stress (Fig. 3h) and a more gradual transition among the elastic, yielding and post-yielding regions. As the fiber dries under load, the formation of β-sheets is indicated by a sudden increase of stress corresponding to a stiffening of the fibers, which can be measured over time as the fibers dehydrate and hydrogen bonds form (Fig. 3i, blue line). When the load is released, the fibers retain their stretched form with a ~ 94 % of fixity yield (R_f), which is calculated as the ratio between the total strain applied ($\varepsilon_{tot} = 80\%$) and the residual strain ($\varepsilon_r \sim 77\%$). After rehydration, the recovery efficiency of the fibers reached values close to 100%, which were likewise confirmed after additional stress-strain cycles (Fig. 3h).

Due to the long-range order of its hierarchical fibrillar structure, the engineered SM material, in its dry state, has a tensile strength (137.18 ± 1.03 MPa) and a Young's modulus (4.18 ± 0.10

GPa) one order and two orders of magnitude higher, respectively, when compared to other reported water triggered shape memory (WTSM) materials (Supplementary Table 1 and Supplementary Fig. 10). These values are in the same order of magnitude of Nylon 6,6 and worm silk fibers.[35,36] Upon hydration, the engineered material tensile strength of 14.94 ± 0.46 MPa is still one order of magnitude higher compared to the reported WTSM materials and in the same order of magnitude of polyurethane elastomers used in the textile industry.[37]

The role of the anisotropic organization of the protofibrils in conferring such high mechanical properties to the keratin fibers was supported by comparison with isotropic thin films obtained from the same extracted keratin solution (Supplementary Fig. 11). The latter showed dramatic decrease in tensile strength (greater than an order of magnitude) and strain to failure (two orders of magnitude), with no plastic deformation attributed to the α-helix-to-β-sheet transition.

4D Printing of Hydration-Responsive Shape Memory Architectures

The processability of the keratin dope was further extended to 3D printing technology for the fabrication of more complex SM architectures that featured the 1-way shape memory modality. Basic geometries could be fabricated by extruding the protein dope into a hydrogel,[38] serving both as supporting and coagulation bath (Fig. 4a). The shear thinning properties of the dope allowed for the use of small-diameter extrusion needles, enabling the production of 3D printed volumes with small textural features in the range of about 50 microns (Fig. 4b). As shown by the birefringence patterns obtained from polarized light microscopy, the alignment of the keratin protofibrils follows the 3D printing extrusion pathway, thus leading to highly ordered architectures, featuring inherent structural hierarchy that spans from the molecular up to the macroscopic level (Fig. 4c).

After the 3D printing process, fixation of the permanent shape requires the formation of the disulfide bridges via H_2O_2-induced oxidation. Before this oxidative step however, the 3D printed objects are mechanically stable without the supporting bath, and therefore can be

plastically manipulated. As reported in Fig. 4d, a star-shaped origami was fabricated by manually folding a 3D printed squared sheet and permanently fixing its new configuration in a solution of H_2O_2 and NaH_2PO_4. This two-steps fabrication process allows for the tailoring of 3D printed basic geometries to achieve permanent architectures with a higher degree of complexity (Fig. 4e).

As with the wet-spun fibers, the 3D printed objects have moisture-responsive shape memory properties. The star-shaped origami architecture was chosen to illustrate the efficiency of the SM mechanism in operating rather sophisticated geometrical transformations. When hydrated, the 3D printed origami is malleable and can for instance be unfolded and arbitrarily reshaped as a rolled-tube (Fig. 4f, left). As it dries, the squared sheet loses its malleability and is locked in its new temporary shape. Recovery of the star-shaped origami architecture is then triggered by rehydration, a process which occurs in the second time scale, due to the high surface-to-volume ratio that causes quick exposure of the bulk keratin to water (Fig. 4f, right). To regain the original origami shape, the squared sheet first opens up through an unrolling process and then correctly refolds through a cooperative bending process of the edges.

Overall, we have developed a hierarchically structured fiber based on fibrillar keratin, thus possessing unique shape memory properties and high mechanical stability. Using a two-stage 3D printing process, where in the first phase we allow the protein to coagulate and in the second stage we cross-link them through an oxidative treatment, we can print fibers into complex shape memory textile materials. This new material, resourced in a sustainable manner, will allow the manufacturing of biodegradable smart textile, like body-adaptable garments or strain-energy absorbing gears.

Outlook

Herein, we proposed a bioinspired design for the fabrication of a biocompatible and hierarchically structured shape memory material from waste keratin. The unique combination

of its high mechanical performances and its SM effect makes the engineered material suitable for the design of actuators finding application in the smart textile industry. Its biocompatibility and body-affinity can be leveraged to replace oil-based polymers for the engineering of strain responsive and body-adaptable apparel. The processability of the material through additive manufacturing platforms allows for the production of complex architectures with structural features in the micron range, making the material suitable for a vast range of bioengineering applications.

Figure Legends

Figure 1 | Keratin extraction protocol and nematic phase formation. a) Schematic depicting the hierarchical structure of wool keratin and the α-helix-to-β-sheet transition feasible under strain. b) Schematic representing the extraction protocol to obtain fibrillar keratin in solution from wool hair. c) Picture of the extracted keratin solution (left), Cryo-TEM micrograph of a ×20 diluted solution of the keratin dope using a 8 M LiBr water solution and showing keratin intermediate filaments (center), Cryo-TEM micrograph zoomed-in showing the hierarchical inner structure of intermediate filaments composed of protofibrils (Similar results were obtained for n = 6 independent Cryo-TEM samples). d) SAXS profile of the scattering vector q obtained from the extracted keratin solution inside a quartz capillary (1.5 mm diameter) and related 2D SAXS pattern (inset), both indicating the nematic phase ordering of keratin under shear and spatial constraint. e) Schematic showing ordering of the keratin protofibrils triggered by the charge screening effect, upon addition of NaH_2PO_4. f) Background subtracted SAXS profiles of the keratin solution showing a shift towards higher q upon addition of NaH_2PO_4, thus tighter packing of the keratin nematic phase. g) Background subtracted SAXS profiles (left) and 2D SAXS scattering patterns (left) of the keratin solution showing peak narrowing upon addition of NaH_2PO_4, (right) and, thus, alignment of the keratin protofilaments along the capillary axis. h) Rheology measurements of the keratin dope showing increase of viscosity and shear thinning property upon increase of NaH_2PO_4 concentration (n = 3 independent batches tested per experimental condition; Data are presented as mean values +/- SEM). i) Picture of fibers directly drawn from the keratin dope containing NaH_2PO_4 in 40 mM concentration.

Figure 2 | Keratin fiber spinning and structural characterization. a) Schematic representing the transition from the keratin nematic phase to the anisotropic fiber. b) Picture of a ~ 100 m long continuous keratin fiber. c) SEM micrograph of a keratin fiber showing its fibrillar structure (Similar results were obtained for n = 6 independent SEM samples). d) Zoom-in of both edge and cross-section of the fiber, showing the fibrillar structure of the fiber core. e) Polarized light microscopy image showing the anisotropic birefringence of the fiber (Similar results were obtained for n = 3 independent fiber samples). f) Polarized Raman spectroscopy in the amide I region (1620-1700 cm^{-1}) of a single keratin fiber oriented parallel to both laser and analyzer (black), perpendicular to both laser and analyzer (grey), parallel to the laser and perpendicular to the analyzer (light-grey) and perpendicular to the laser and parallel to the analyzer (green). g) 2D WAXS scattering pattern obtained from a bundle of keratin fibers oriented perpendicular to the x-ray beam and parallel to the meridian axis of the detector, with

white arrows pointing at the meridional and equatorial reflections. The black bars are non-detecting areas corresponding to the gaps between the detector plates. h) One-dimensional WAXS scattering profiles showing a maximum at 5.05 Å and a shoulder at 5.15 Å, belonging to the α-helix pitch projections (meridian), and a maximum at 9.65 Å belonging to the spacing between adjacent α-helix axes (equatorial) (left); i) Schematic of the fiber structure composed of coiled-coils aligned along the fiber axis. A fraction of α-helices is denaturated into uncoiled peptide chains and β-sheets during fiber spinning.

Figure 3 | Shape memory effect in keratin fibers. a) Schematic of the keratin protein secondary structure rearrangement during the α-helix-to-β-sheet transition under strain (Schematic redrawn with permission from Ref. 13 (Fig. 5"). b) Stress-strain plot of a single keratin fiber showing the three typical regions describing the α-helix-to-β-sheet transition as function of strain (Representative of n = 3 curves). c) Schematic depicting the hydrogen bonds network responsible for the formation of the keratin secondary structures and the change of the bond orientation within the fiber as keratin rearrange from the α-to-β structure. d) Strain-dependent Raman spectroscopy of a single keratin fiber. The black and the red solid line represent the experimental and the sum of the calculated peaks, respectively, while the dashed lines belong to the single calculated peaks e) Schematic describing the formation of β-sheets from coiled-coils and their alignment along the fiber axis, (left); 2D WAXS pattern of the strained fiber, with white arrows pointing at the characteristic equatorial reflections (right). f) Schematic representing the water-triggered shape memory mechanism. g) Screenshots taken from video 1 and illustrating the water-triggered shape memory behavior of the keratin fiber bundle. For clarity purposes, each state of the yarn during the process is labeled as follow: wet and relaxed (state A), wet under load (state B), dry under load (state C), dry and relaxed after stretch (state D), and wet and relaxed after recovery (state A'). h) Stress-strain plot of a keratin fiber yarn undergoing multiple water-triggered shape transitions. i) Sress-time plot of the full cycle 2. In both cases labels A, B, C, D and A' correspond to the same yarn states described in figure 4g.

Figure 4 | Shape memory effect in 3D printed architectures. a) Photograph illustrating the 3D printing process using the extracted keratin as an ink (401.7 mg/mL, NaH_2PO_4 40 mM) and a pluronic solution (25% v/w) as both a supporting and coagulation bath (left). Images of basic 3D printed keratin architectures such as ring, flat star and flat stripe (right). For image clarity,

376 the keratin solution was dyed with Rhodamine B to give the objects a purple color. b) SEM
377 micrographs showing the 3D structure of the keratin architecture composed of aligned and
378 stacked fibers deposited according to a rectilinear pattern (Similar results were obtained for n
379 = 4 independent SEM samples). c) Polarized light microscopy images of a rectilinear 3D printed
380 pattern showing the anisotropic birefringence for aligned single fibers (Similar results were
381 obtained for n = 3 independent 3D printed grids). Scale bar 400 μm. d) Sequence of photographs
382 illustrating the post 3D printing process implemented to tailor the shape of basic 3D printed
383 structures. Specifically, a star-shaped origami is obtained from a flat square, which is first
384 sandwiched between two paper sheets and then folded into the desired origami architecture, to
385 be finally fixed in a H_2O_2 and NaH_2PO_4 water solution. e) Photographs of a star with bended
386 arms (top) and spirals (bottom) obtained by post 3D printing processing. (scale bars 1 cm). f)
387 Photographs describing the water-triggered SM effect in 3D architectures which is illustrated
388 with a star-shaped origami.
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Methods

General methods. All reagents were commercially available and used without further purification unless otherwise stated. Angora wool was purchased from R. H. LINDSAY COMPANY (Dorchester, Massachusetts, USA), Lithium bromide (LiBr), hydrogen peroxide (H_2O_2), sodium dihydrogen phosphate (NaH_2PO_4), Rhodamine B and 1,4-dithiothreitol from (DTT) from Sigma Aldrich, while Pluronic F127 Surfactant Prill was purchased from BASF. Dialysis cassettes Slide-A-LyzerTM with cut-off of 3.5 KD 0.1-0.5 mL were purchased by Thermo Fischer Scientific. UV-vis spectra were recorded using Agilent Cary 60 UV/VIS spectrophotometer and a 1 cm path quartz cuvette, while data collection and analysis were carried out using Cary WinUV v2 software. Raman spectroscopy was performed using a XploRA Hyperspectral Darkfield Raman Microscope and using a 785 nm excitation laser, while polarized Raman spectra recorded using a Horiba LabRam Evolution using a 633 nm excitation laser. Raman spectroscopy data collection and analysis were carried out using LabSpec v6.4.4 software. Rheology measurements were performed on a Ares-G2 rheometer using a conical geometry of 40 mm diameter, while data collection and analysis were carried out using Trios v4.4.1 (TA instruments) software. Circular dichroism spectra were recorded using a Jasco J-815 CD Spectrometer and a 0,5 cm path quartz cuvette, while data collection and analysis were carried out using SpectraManager v2 software.

Wool grinding protocol. First, wool was washed with ethanol for 40 hours using a continues Soxhlet extraction system, rinsed with water and allowed to dry at room temperature. Successively, wool fibers were manually cut into shorter segments (approximately 5 mm) and grinded to micron-size particles using a Retsch PM100 planetary ball mill. 20.5 g of cut wool were placed in a 250 mL stainless steel jar together with stainless steel balls (90 ml, 5 mm in diameter) and allowed to grind at 450 rpm for 3 hours with intervals of 5 minutes every 20 minutes.

Keratin extraction protocol. Under N_2 atmosphere, wool powder (9.5 g) was suspended in a water solution (150 mL) of lithium bromide (8 M) and DTT (0.100 mM), and the reaction allowed to vigorously stir at 90 °C for 36 hours. Afterwards, wool residue was collected by hot filtration under negative pressure and the solution allowed to cool down at room temperature. NaCl (2,30 g, 25 g/mL) was added portion wise under stirring and the solution successively stored at 4 °C for 12 h to obtain a heavy keratin colloidal phase via phase separation. The colloidal protein phase formed was separate from the solution via centrifugation (3000 rpm, 4 °C) and collected as a yellowish viscous liquid (10.31 mL). The obtained keratin solution was dialysed against water (3 × 2 L) over 2 days using a dialysis cassette with a cut-off of 3.5 KD and finally freeze dried to obtain a white solid. Based on the Bradford assay, the extracted keratin solution concentration was calculated based on the Bradford assay to be 401.7 ± 15 mg/mL. (total yield 43.6 %).

Scanning electron microscopy (SEM). Samples were deposited on a SEM stub (12.5 mm in diameter) covered with carbon tape and successively sputter coated with Pt/Pd (10 nm thickness) using an EMS 200T D dual sputter coater. SEM micrographs were taken using a Zeiss Ultra Plus Field Emission Scanning Electron Microscope using an electric high tension (EHT) of 2 kV and a SE2 detector. Data collection and analysis were carried out using SmartSEM v05.06 image processing software

3D Printing. The Cellink (BIO X Model) 3D printer is used as 3D printing platform. The keratin dope is extruded at 40 °C, under a pressure of 90 kPa and through a 36 gauge needle moving at a speed of 8 mm/s. Pluronic F127 (25% m/v water solution) is used both as coagulating and supporting baths. To help visualize the object structure during printing, Rhodamin B is added in the keratin dope as tracer (Pink color). After 3D printing, Pluronic is removed by washing with a cold (~ 0 °C) 0.4 M NaH_2PO_4 solution. Oxidative fixation is carried out with a H_2O_2 (1% v/v) water solution over 1 hour.

Cryo-transmission electron microscopy (Cryo-TEM). Cryo-TEM analysis was performed as follows: A droplet (5 μL) of the sample solution was placed on hydrophilised (plasma treatment) Quantifoil 'holey' grids R 2/1 Cupper. The excess fluid was blotted off to create an ultra-thin layer (typical thickness: 200-300 nm) of the solution, which spans the holes of the support film. The prepared samples were vitrified by quick immersion of the grids into liquid ethane at its freezing point (-184 °C) using a Gatan Cryoplunge 3 system. The vitrified sample grids were transferred under liquid nitrogen into the FEI Tecnai Arctica CryoTEM equipped with a 200kV Schottky field emission gun. Microscopy was carried out at -175 °C sample temperature using the microscope's low dose protocol at calibrated primary magnifications 23.5 k or 39 k. Data collection and analysis were carried out using ENAM v2 image processing software.

Mechanical tests. Samples were prepared according to the ASTM D3822, by using acrylic tensile test frames with 25 mm gauge length and attaching single fibers or fiber bundles with epoxy resin. Sample were tested using Instron 5566 tensile tester equipped with a 2525 Series Drop-through 10 N load cell and pneumatic grips. Data collection and analysis were carried out using Bluehill v3 software.

Small-angle X-ray scattering (SAXS). Experiments were performed in the 4C SAXS beamline of Pohang Accelerator Laboratory (PAL). Keratin SAXS samples were transferred to quartz capillaries (with 1.5 mm of outside diameter and 0.01 mm wall-thickness) (Charles Supper Company, Natick, MA) and centrifuged for 4 hours at 2500 rpm. The scattering data were acquired with X-ray beam illumination at sample-to-detector distance (SDD) = 5 m. The samples were measured for 60 sec to prevent radiation damage on samples. 2D SAXS data were averaged into 1D curves of scattering intensity versus q. The backgrounds were set as a polynomial function that passed through the scattering minima of each SAXS curve.

Wide-angle X-ray scattering (WAXS). data were acquired using the SAXSLab instrument (CMSE X-ray facility (Massachsetts Institute of Techniology, Cambridge MA, USA) equipped

with a Rigaku 002 microfocus X-ray source and Osmic staggered parabolic multilayer optics. A wavelength of 45 keV (0.66 mA, 0.0275 nm) was chosen. A DECTRIS Pilatus3R 300K detector was used and positioned 109.1 mm from the sample. The beam size at the sample was approximately 900 × 900 μm². Samples were prepared by gluing a bundle of parallel fibers (~ 40) on an acrylic frame and positioning the latter perpendicular to the beam and with the longitudinal fiber axis parallel to the meridian axis of the detector. Data collection and analysis were carried out using SAXSGUI v2.23.09 and RAW v1.5.1.

Statistics and Reproducibility. Experimental errors are calculated as standard error of the mean, with "n" referring to the number of analyzed samples and reported in the respective figure legends.

Data Availability

All produced data that support this study are included in this published article and its supplementary information files. Data points for the mechanical tests are provided as source data files. Additional data are available from the corresponding authors upon request.

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Authors Contributions

The work was conceived and designed by L.C and K.K.P. L.C. implemented the keratin extraction, fiber spinning and 3D printing protocols. J.L., M.C.C. and K.S. conducted the SAXS experiments and related data analysis and interpretation. G.M.G. and Q.L. carried out rheological measurements and related data analysis. C.O.C., G.M.G. and L.C. carried out

tensile tests. L.C. conducted Raman spectroscopy, polarized light microscopy, SDS-Page, WAXS, CD, SEM, Cryo-TEM and related data analysis and interpretation. S.C. and R.G. designed the 3D printed structures. L.C. wrote the manuscript. Q.L., GMG., C.O.C., K.S. and K.K.P. edited the manuscript. All authors discussed the results.

Competing interests

The authors declare no competing interests