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Controlled De-Cross-Linking and Disentanglement of Feather Keratin for Fiber Preparation via a Novel Process

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ABSTRACT: Pure protein fibers were fabricated from chicken feathers via a potentially green process. In the last several decades, efforts have been made to produce keratin-based industrial products, especially fibers. However, the methods of producing keratin fibers directly from chicken feathers could not be repeated. In this research, protein fibers from chicken feathers were developed using chemicals that could be either derived from renewable resources or facilely recycled. Backbones of keratin were preserved after cleavage of disulfide bonds using cysteine. Sodium dodecyl sulfate (SDS) was applied to dissolve keratin for spinning. Increasing SDS concentration intensified the ordered conformation of keratin, first increased and then decreased the viscosity of solution, suggesting continuous disentanglement of keratin molecules and enhancement in inter- and intramolecular electrical repulsion. Diameters of the obtained fibers as small as 20 μm also proved good drawability of the keratin solution. Change in crystallinity indices was found to be consistent with that of tensile properties of the keratin fibers. In summary, regenerated fibers were successfully produced as linear keratin with preserved backbones that could be untangled and aligned in a controlled manner.

KEYWORDS: Regenerated keratin fibers, Chicken feathers, Surfactant, Controlled disentanglement, Viscosity

INTRODUCTION

Global fiber production in 2012 approached 85.8 million tons, of which approximately 50.6 million tons were synthetic fibers and about 30 million tons were cotton fibers.1 Synthetic fibers are not sustainable because of limited petroleum reserves and rising oil price, while production of cotton, the major natural fiber, has been decreasing. Therefore, to satisfy the increasing global consumption of fibers and to resolve the problem of limited resources, it is necessary to develop fibers from alternative resources with large availability at low price.

Chicken feathers could be prospective resources to produce regenerated protein fibers. The Unite States poultry industry produces more than 4 billion pounds of chicken feathers each year.2 Some of the feathers are autoclaved or hydrolyzed and then used as animal feed with low nutritional value,5 and the rest are disposed through landfills.

Developing regenerated keratin fibers could not only provide new sources for the fiber industry to alleviate the fiber shortage but also add value to the poultry industry and address related environmental concerns. Chicken feathers contain about 90 wt % of keratin. As small linear proteins with a few bulky side groups and molecular weight higher than 10 kDa, feather keratin meets the molecular requirements for fiber spinning.4 Keratin has about 7% cysteine, which could serve as cross-linking sites to form water-stable fibers.5

To the best of our knowledge, no efficacious method has been developed to produce regenerated keratin fibers, although relevant research could date back several decades. In the 1940s, regenerated keratin fibers had been fabricated via wet spinning of protein—surfactant complexes in the laboratory.6,7 However, mechanical properties of the fibers were not reported. Nevertheless, we tried the methods and found that the results could not be repeated, and did not find any other reports regarding successful repetition of the methods as well. A recent paper reported successful electrospinning of soy protein with SDS.8 In the 2000s, the U.S. Department of Agriculture (USDA) launched projects to transform chicken feathers into industrial products, especially fibers. However, we did not find
reports about related fibrous products. The latest report
regarding spinning of pure regenerated keratin fibers was in
2008. Fan dissolved feather keratin in ionic liquid for wet
spinning. However, the obtained fibers showed tensile strength
as low as 0.2 g per denier.

Regarding composite fibers with keratin as one of the
components, keratin and poly(vinyl alcohol) (PVA) composite
fibers have been produced via wet spinning. Pure keratin
fibers had advantages of sustainability and renewability
compared to keratin/PVA fibers. Due to the high percentage
of PVA incorporated and usage of toxic cross-linkers, the
keratin/PVA fibers were unsustainable, nonrenewable, and
had limited industrial potential. In the composite fibers, PVA
accounted for 33–67% of the total fiber weight. Therefore, the
fibers were highly petroleum-dependent and thus were
non sustainable or nonrenewable and had potentially higher
cost as the oil price increased. In addition, because 33
nonsustainable or nonrenewable and had potentially higher
environmental and health problems.

In this research, linear keratin with preserved backbones
was obtained by breaking disulfide bonds connecting the keratin
networks in chicken feathers under mild conditions. Controlled
disentanglement and alignment of the keratin molecules by
incorporating SDS led to dissolution of water-insoluble keratin
in aqueous solution with good spinnability. Effect of SDS was
verified via viscosity and conformational studies. Influence of
different coagulation baths on mechanical properties was also
investigated. Retention of backbones during extraction and
unraveling of linear keratin with SDS in spinning dopes ensured
formation of pure keratin fibers with good fineness and
mechanical properties. The whole process could be considered
as green because the major chemicals could be produced from
renewable resources and the swelling agent could be recycled.

# EXPERIMENTAL SECTION

## Materials.
Chicken feather barbs were provided by Featherfiber
Corporation, Nixa, MO. Sodium dodecyl sulfate (SDS, 99.0%) was
purchased from Fisher, Inc., San Francisco, CA, and used as
supplied by Oakwood Chemical, Inc., West Columbia, SC. Coomassie
Brilliant Blue R-250 (Proteomics grade) was purchased from EMD
Chemicals Inc., Gibbstown, NJ. Chemical reagents used in SDS-
PAGE analysis, including LDS sample buffer (4×), NuPage 20× MES
running buffer and NuPAGE 4–12 Bis-Tris gel, were purchased from
Invitrogen, Inc., Grand Island, NY. Cysteine (98.0%) was
purchased from Amresco, LLC, Solon, OH. The purity of chemicals
was considered in all the calculations of concentrations.

Major chemicals involved in the process were either from renewable
resources or can be reused. Urea could be recycled after extraction of
keratin. Cysteine is a standard amino acid with a thiol group that
shows strong reducibility. It is an environmentally benign reducing
agent and can be commercially produced via fermentation. SDS is
synthesized by treating lauryl alcohol with sulfur trioxide gas, while the
lauryl alcohol is usually derived from vegetable oils, such as coconut oil
or palm oil by hydrolysis.

Controlled Cleavage of Disulfide Cross-Links in Feathers.
Critical conditions, such as concentration of cysteine, pH, and
treatment time were used to study treatment conditions based on yield
and molecular weight. The weight ratio of 8 M urea solution to
chicken feathers of 17:1 was used to completely immerse chicken
feathers, and the treatment temperature was 70 °C. To study the effect
of reductant, cysteine concentrations of 1%, 5%, 10%, and 20% on
the weight of weight of chicken feathers were selected. The pH was 10.5, and
treatment time was 24 h. The pH of feather dispersion was adjusted
using 50% NaOH solution. Different periods of treatment time, 3, 6,
12, and 24 h, were selected, while the cysteine concentration was 10 wt
% and pH was 10.5. To study the pH effect, four sets of pH, 6, 9, 10.5,
and 11.5, were used, while the cysteine concentration was 10 wt % and
the treatment time was 12 h. After optimization based on yield and
molecular weight distribution, conditions of 10 wt % of cysteine, 12 h, and
pH 10.5 were used for extraction of keratin for study on spinning
do and wet spinning.

After treatment, dispersion of feathers in 8 M urea was centrifuged
at 10000 rpm for 20 min to precipitate undissolved feather residues.
The supernatant was adjusted to pH 4 using hydrochloric acid and
sodium sulfate to precipitate dissolved keratin. The keratin precipitate
was washed three times with distilled water under centrifugation of
10000 rpm for 20 min. The collected keratin was dried at 50 °C.

SDS-PAGE. About 1 mg of extracted keratin from different
extraction conditions was dissolved in 100 μL of NuPAGE LDS
sample buffer (1×), heated at 70 °C for 10 min, and left standing at
room temperature for 2 h. The solution was vortexed prior to loading.
Each sample of 10 μL was loaded into an individual slot of the gel.
After electrophoresis, the gel was stained with Coomassie Brilliant
Blue R-250 for 2 h at room temperature. The gel was then
destained using 10% acetic acid until a clear background was observed.

Circular Dichroism (CD).
Keratin was dissolved in 0.3 M sodium carbonate–sodium bicarbonate buffer at pH 9.5 to enable disentanglement of polypeptides. The spinning
dope was prepared by dissolving 30% extracted keratin and 10% SDS (based on weight of keratin) in 0.3 M sodium carbonate–sodium bicarbonate buffer at pH 9.5. The spinning dope was allowed to age for 24 h at room
temperature to enable disentanglement of poly peptides. The spinning
do was deposited in a water bath at 90 °C for 1 h before spinning. The
fibers were then drawn manually in three types of coagulation bath, i.e.,
10% sodium sulfate solution and 10% acetic acid (NaSO4/acetic acid),
10% ethanol and 10% acetic acid (ethanol/acetic acid), 10% methanol and 10% acetic acid (methanol/acetic acid). The fibers were
washed in distilled water, dried under ambient conditions, heated at
150 °C for 2 h, drawn manually twice, and annealed at 120 °C for 1 h.
The fibers were balanced in 21 °C and 65% relative humidity for 24 h
prior to any test.

Morphological Analysis.
Morphological analysis of fibers was studied using a scanning electron microscope (SEM, S3000N, Hitachi, Inc., Schaumburg, IL). Keratin fibers were sputter-coated with gold/ palladium and observed at a voltage of 15 kV.

Tensile Properties.
Tensile properties of the keratin fibers were investigated in
terms of denier, which is the weight of 9000 m of fibers in grams. The
weight of known lengths of keratin fibers was measured to calculate

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the denier of fibers. Tensile properties of fibers in terms of breaking tenacity and breaking elongation were tested using an Instron tensile testing machine (Norwood, MA) according to ASTM standard D-3822. In the test, a gauge length of 1 in. and crosshead speed of 18 mm min\(^{-1}\) were used. For each condition, about 30 specimens were tested for each fiber sample. The wet strength of keratin fibers was determined immediately after immersing the fibers in water at room temperature for 30 min.

**Crystallinity Analysis.** X-ray diffraction studies were carried out on raw chicken feathers, extracted keratin powder, keratin fibers from sodium sulfate/acetate acid, methanol/acetate acid, and ethanol/acetate acid coagulation bath. The data were obtained using a Rigaku D/Max-B X-ray diffractometer with Bragg–Brentano parafocusing geometry, diffracted beam monochromator, and conventional copper target X-ray tube set (\(\lambda = 1.54 \) Å) to 40 kV and 30 mA at 26 °C. Diffraction intensities were recorded within 2\(\theta\) ranging from 3° to 40° at a scan speed of 0.05° per second. The crystallinity index (C.I.), indicating the relative crystallinity degree of fibers, has been long used to characterize keratin fibers such as wool and was calculated using the following empirical eq14

\[
\text{C.I.} = \frac{I_0 - I_{14}}{I_0}
\]

where C.I. is the crystallinity index; \(I_0\) is the maximum intensity of crystal lattice diffraction with 2\(\theta\) at around 9°, and \(I_{14}\) is the minimum diffraction intensity with the 2\(\theta\) at around 14°. In general, a higher C.I. value indicates higher crystallinity of the fiber sample.

**Statistical Analysis.** All the data obtained were analyzed by the one-way analysis of variance with the Scheffe test with a confidence interval of 95%. A \(p\) value smaller than 0.05 indicated a statistically significant difference. Standard deviations are shown by the error bars in the figures, and the data in the figures labeled with different numbers or characters indicate significant differences among them.

### RESULTS AND DISCUSSION

**Controlled Breakage of Disulfide Cross-Links.** Dissolution process of chicken feathers using 8 M urea and reducing condition included swelling of keratin macromolecules by urea and breakage of disulfide bonds by cysteine. Difficulty in dissolving chicken feathers stemmed mainly from their high cross-linking degree, attributed to 7–10% of cysteine in keratin, and hydrophobic interactions due to approximate 40% of amino acids with hydrophobic side groups. In the 8 M urea solution, urea concentrated on the surface of protein, disturbed inter- and intramolecular hydrogen bonds, weakened the hydrophobic interaction between polypeptides and consequently led to exposure of more polypeptide chains of keratin to the solvent. Thus, the reductant could react with disulfide bonds that used to be buried inside the peptide assembly. The keratin extracted under swelling and reducing conditions in this research was water insoluble.

Figure 1 demonstrates molecular weights of keratin extracted from different conditions. From lane 1 to 4, all the keratins showed dark bands at 10 and 20 kDa. In lane 1, the dark smear between 28 and 188 kDa indicated that most of the extracted proteins originated from slightly alkaline hydrolysis, which broke the backbones in a nonspecific manner. Dull bands around 30 and 40 kDa appear in lanes 2 and 3, while none appear in lane 4, suggesting breakage of more disulfide bonds with a higher content of cysteine. From lanes 5 to 8, a gradual transition of molecular weight distribution from higher molecular weights to lower molecular weights could be attributed to intensified hydrolysis of keratin at pH 10.5 as time prolonged. The bands at 10 kDa in lanes 7 and 8 are much weaker than those in lanes 5 and 6, suggesting a low amount of extracted polypeptides or hydrolysis of polypeptides at this molecular weight to water-soluble small peptides. Lane 9 represents condition with pH 6, which is much lower than the pK\(_a\) 8.2 of the thiol group in cysteine, while lanes 10, 11, and 12 represent the pH of 9, 10.5, and 11.5, respectively. In lane 9, there are strong bands at 10 kDa, while lanes 10, 11, and 12 have much stronger bands at 20 and 30 kDa. Moreover, at high pH can attack the disulfide bonds in feather keratins. At pH 6, undeprotonated thiol groups dominated. Cross-links in the keratin could not be completely broken, and therefore, keratin could not be dissolved.

Percent yield of extraction shown on the top of each lane in Figure 1 was also critical because the ultimate goal was to produce fibers from keratin on an industrial scale. Considering the results of yield and molecular weight, the condition of cysteine of 10 wt %, pH 10.5, and treatment time of 12 h as shown in lane 11 was selected for extraction of keratin in this study.

**Effect of SDS on Conformation of Keratin.** SDS played a critical role in keratin dissolution to enhance disentanglement and alignment of molecules and thus endowed the keratin solution with spinnability. Spinnability, indicating the capability of polymer solution to elongate irreversibly under stretching in one direction, could be improved if the entangled polymers were unraveled and became aligned, while a small portion of polymers were left hooked to each other.

Conformations of keratin molecules with different SDS concentrations in solution are illustrated in Figure 2. All of the spectra with strong negative absorption at 200 nm and humps at around 215–218 nm show good approximation to typical spectra of unordered conformation of polypeptides.17 The spectrum gradually weakened as SDS concentration increased from 5 to 40 wt %, indicating less random conformation of keratin molecules. At a higher concentration of SDS, keratin molecules tended to associate with more SDS and carried a stronger negative charge thereafter. The increased SDS content further dissociated the hydrophobic interaction among keratin
molecules, as well as intensified inter- and intramolecular repulsion. Consequently, the individual keratin molecules were forced to straighten, became more rigid, and assembled into more organized structures. By increasing the SDS concentration, continuous disentanglement and straightening of keratin molecules throughout the dissolution process were proved by the conformational change.

Figure 3 demonstrates that viscosity of keratin dispersion or solution increased as the concentration of SDS increased, and the maximum viscosity was achieved when the SDS concentration reached 10 wt %. A further increase in SDS concentration decreased viscosity to around one-sixth of the peak value. The trend was in accordance with several reports regarding the interaction between SDS and proteins or other polymers.18

To explain the transition of viscosity shown in Figure 3, the dissociation process of keratin is proposed in Scheme 1. Without SDS in the keratin dispersion, the hydrophilic chains of keratin surrounded the hydrophobic domains in a highly coiled conformation to segregate them from water, as shown in Scheme 1a. Interaction among the coils were remarkably weak due to the limited contact area among the coils. The viscosity of less than 1 mPas was mainly attributed to interaction among water molecules. Viscosity of the dispersion increased slowly as SDS concentration increased from 5 to 8.75 wt % as shown in Figure 3. SDS disrupted keratin coil conformations by “dissolving” hydrophobic domains and liberating hydrophilic portions in the keratin molecules. After the C12 alkyl tails of SDS associated with hydrophobic domains in keratin coils via hydrophobic interaction, the hydrophilic portions used to embed the hydrophobic domains in the coils could contact water more extensively. Therefore, the whole keratin molecules in the coils gained more surface negative charge attributed to the attached sulfate groups. As a result, the volume of coils was enlarged, and interaction among them was also strengthened, although at a relatively slow rate. Subsequently, viscosity increased steeply as SDS increased from 8.75 to 10 wt %. Here, keratin started to dissolve and became solution. Extensive liberation of keratin molecules from coils may occur, leading to a dramatic increase in contact area among keratin backbones and thus considerably strengthening the molecular interaction. At the same time, a certain degree of molecular entanglement may still exist and could also contribute to the molecular interaction, as demonstrated in Scheme 1c. However, further addition of SDS from 10 wt % rapidly decreased viscosity. It could be inferred that the remaining entanglement among keratin molecules may be completely dissociated by excess SDS, while electrical repulsion among molecules was increasingly enhanced because more SDS could join existing micelles on the surface of molecules. Breaking of entanglement and enhancement in molecular repulsion significantly weakened molecular interaction as the contact area of liberated molecules was further reduced. At this concentration, intensified interaction among SDS micelles on keratin molecules may dominate the decrease in viscosity, as illustrated in Scheme 1d.

In fiber spinning, low SDS concentration resulted in poor alignment of molecules and thus unsatisfied drawability of polymers, while high SDS concentration that led to weak interaction among molecules could result in poor physical properties of fibers. Therefore, SDS of 10 wt %, at which concentration the keratin molecules showed the strongest
interaction and appropriate disentanglement, was selected for fiber spinning.

**Wet Spinning of Keratin Fibers.** Figure 4 shows regenerated keratin fibers from chicken feathers on the left and natural wool fibers on the right. The regenerated keratin fibers resembled natural wool fibers in terms of various sensory properties, such as length, fineness, and crimp.

Figure 5a–c illustrate morphologies of regenerated keratin fibers coagulated in solutions of sodium sulfate/acetic acid, methanol/acetic acid, and ethanol/acetic acid, respectively. All the fibers had diameters of around 20 μm, reflecting good spinnability of keratin. The fiber coagulated in sodium sulfate/acetic acid had a coarser surface than that from the alcohol-containing bath. The difference was ascribed to the relatively higher solubility of hydrophilic keratin in the aqueous electrolyte solution than in the organic solvent. Keratin molecules in the sodium sulfate solution were inclined to diffuse into the bath, and therefore, the ordered alignment of polymers in the spinning dope might be disrupted, resulting in an uneven fiber surface. However, keratin molecules with much lower solubility in alcohol tended to integrate within molecule bundles to reduce exposure of molecules to alcohol and thus better retain their ordered arrangement in the spinning dopes. Therefore, keratin fibers from the alcohol coagulation bath showed much smoother surfaces than those from the electrolyte solution.

**Structure and Property Correlation in Keratin Fibers.** Mechanical properties of the regenerated keratin fibers at dry and wet states are shown in Figure 6. The dry tensile strength of the fibers coagulated in the sodium sulfate/acetic acid solution was approximate 59 MPa, which was significantly lower than 72 and 75 MPa, that of the fibers from the coagulation baths with methanol/acetic acid and ethanol/acetic acid, respectively. There was no significant difference between the strength of the fibers from the two alcohol/acetic acid solutions. All the tensile properties were remarkably lower than that of the raw feathers, which was 203 ± 74 MPa as reported. The wet tensile strength of fibers from the three coagulation baths of around 30 MPa was not significantly different from each other and was still much higher than that of the regenerated soyprotein fibers, which was around 19 ± 3 MPa. The better wet strength of keratin fibers could be attributed to the remaining cross-links among the molecules. The dry elongation of the regenerated keratin fibers of about 5% and the wet elongation of about 25% were much lower than those of other regenerated protein fibers.

The coagulation baths showed critical effects on the mechanical properties of the wet-spun keratin fibers. The pH of all three coagulation baths with the same 10% acetic acid around 2.5 was lower than S, the pI of keratin. Therefore, the effect of the acidic solidification of keratin was similar in all three conditions. SDS in the keratin solution could diffuse into the infinite coagulation bath and resulted in decreased solubility of keratin. In the sodium sulfate solution, sodium and sulfate ions interrupted hydrogen bonds between water molecules and keratin and thus disrupted the hydration layers on the keratin molecules. The hydrophobic interaction could be formed among keratin molecules and lead to solidification of keratin fibers. Regarding the alcohol coagulation bath, alcohol could induce conformational transition of protein to β-sheet, which could be beneficial to mechanical robustness of protein fibers.
Methanol was widely used in lab-scale wet spinning of protein fibers. The ethanol coagulation bath, which endowed regenerated keratin fibers with similar properties to methanol coagulated fibers, could have potential applications for large-scale production.

With tensile properties of about 75% for natural wool, the obtained keratin fibers have the potential to be used in industries. They could be blended with wool, cotton, or synthetic fibers to make yarns and other industrial products. Furthermore, mechanical properties of the fibers will be improved to meet the industrial standards via many widely used approaches, such as chemical cross-linking and nanoparticle filling in the future.

Figure 7 shows the wide-angle X-ray diffraction profiles of raw chicken feathers, extracted keratin powder, and keratin fibers using coagulation baths of ethanol/acetic acid, sodium sulfate/acetic acid, and methanol/acetic acid, respectively. All the samples showed a typical diffraction pattern of α-keratin with a prominent 2θ peak at 20.2° and a minor peak at 9°, indicating the crystalline spacings of 4.4 and 9.8 Å, respectively. The wider peak area implied smaller crystals in the proteins. Among them, raw feathers with narrower 2θ peaks at 20.2° than the other samples should possess relatively large crystals.

After the original molecular structures were disassembled during extraction and reassembled after fiber spinning and coagulation, the minor peak of raw chicken feathers shifted from around 9.6° to 9.2° of alcohol coagulated fibers and to 9.1° of powder and electrolyte coagulated fibers. A decrease in the minor peak positions indicated increasing spacing between two adjacent lattice planes within the proteins, in other words, less compact molecular structures of the proteins.

As shown in Table 1, the decreasing trend of C.I. was also in accordance with the difference in tensile properties from raw feathers to regenerated fibers from the alcohol coagulation bath to fibers from the electrolyte coagulation bath as shown in Figure 7. The keratin powder showed an even lower C.I. value, indicating even lower crystallinity. It was suggested that the precipitated keratin in either form of powders or fibers could not completely reconstruct the tight arrangement of poly-peptides existing in the raw feathers. It has been proved in the literature that aqueous organic solvent induced a decline in the α-helix and an increase in the β-sheet. With alcohol precipitation, much tighter molecular structures, probably β-sheets, could be partially rebuilt in the protein fibers and result in better mechanical performances.

Table 1. C.I. of Chicken Feathers, Extracted Keratin Powder, and Regenerated Keratin Fibers Using Coagulation Baths Containing Sodium Sulfate/Acetic Acid, Ethanol/Acetic Acid, or Methanol/Acetic Acid

<table>
<thead>
<tr>
<th></th>
<th>feathers</th>
<th>methanol</th>
<th>ethanol</th>
<th>Na2SO4 powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.I.</td>
<td>54.48</td>
<td>32.45</td>
<td>30.95</td>
<td>21.27</td>
</tr>
<tr>
<td>Keratin Powder</td>
<td>19.56</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

Pure regenerated protein fibers have been successfully developed from chicken feather keratin. Keratin with preserved backbones was extracted by breaking disulfide bonds under mild conditions. SDS was incorporated to disentangle and align keratin molecules to endow them with good drawability. SDS enhanced the orderedness of keratin and first increased and later decreased the viscosity of the keratin solution. The wet-spun fibers from keratin–surfactant spinning dopes had diameters as low as 20 μm, which indicated the good spinnability of the keratin solution. The crystallinity indices of fibers from different coagulation baths changed in the same trend as the tensile properties. Cleavage of disulfide cross-links in keratin with retained backbones and dissolution of keratin using SDS was critical to regenerate protein fibers.

The dissolution methods developed in this research will have considerable broad impacts on exploring applications of many highly cross-linked proteins from biofuel and agricultural industries. For example, sorghum proteins are generated from the bioethanol industry in large quantities but with limited...
applications because they are insoluble in common solvents due to the high cross-linking degrees. The dissolution method developed in this research will make the dissolution of sorghum proteins possible. It could be inferred that many available highly cross-linked proteins (e.g., sorghum proteins) by products in biofuel and agricultural industries could be developed into useful industrial products, such as films, sponges, and fibers.

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**Notes**
The authors declare no competing financial interest.

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