JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Collagen Orientation and Leather Strength for Selected Mammals

Katie H. Sizeland,[†] Melissa M. Basil-Jones,[†] Richard L. Edmonds,[‡] Sue M. Cooper,[‡] Nigel Kirby,[§] Adrian Hawley,[§] and Richard G. Haverkamp*,[†]

[†]School of Engineering and Advanced Technology, Massey University, Private Bag 11222, Palmerston North, New Zealand 4442

[‡]Leather and Shoe Research Association of New Zealand, P.O. Box 8094, Palmerston North, New Zealand 4446

[§]Australian Synchrotron, Melbourne, Australia

ABSTRACT: Collagen is the main structural component of leather, skin, and some other applications such as medical scaffolds. All of these materials have a mechanical function, so the manner in which collagen provides them with their strength is of fundamental importance and was investigated here. This study shows that the tear strength of leather across seven species of mammals depends on the degree to which collagen fibrils are aligned in the plane of the tissue. Tear-resistant material has the fibrils contained within parallel planes with little crossover between the top and bottom surfaces. The fibril orientation is observed using small-angle X-ray scattering in leather, produced from skin, with tear strengths (normalized for thickness) of 20−110 N/mm. The orientation index, 0.420−0.633, is linearly related to tear strength such that greater alignment within the plane of the tissue results in stronger material. The statistical confidence and diversity of animals suggest that this is a fundamental determinant of strength in tissue. This insight is valuable in understanding the performance of leather and skin in biological and industrial applications.

KEYWORDS: *collagen, orientation, alignment, leather, strength, SAXS*

INTRODUCTION

The strength of collagen materials is of crucial importance in both medical and industrial contexts. Collagen is the main structural component of skin,[1] leather, and some medical scaffolds.[2] Medical conditions can arise when tissues do not have the required mechanical strength, such as in aneurysms,[3] cervical insufficiency,[4] osteoarthritis,[5] and damaged articular cartilage.[6] In addition, bone is a composite material in which the structure of collagen is considered to be important for bone toughness.[7,8] Strength is also a requirement for collagen-based medical materials such as extracellular matrix scaffolds[2] and processed pericardium for heart valve repair.[9] Leather, which is processed skin consisting mostly of collagen, is produced on a large scale for shoes, clothing, and upholstery,[10] with high strength being a primary requirement for high-value applications.

Factors that have previously been considered as possibly contributing to the strength of collagenous materials include the amount of collagen present, the molecular structure of the collagen (D-spacing, collagen type), the nature of the cross-linking between collagen,[11] collagen bundle size, and collagen orientation. Of these, much attention has been given to collagen orientation. Most collagen tissues are anisotropic, and it is understood that this is a result of the nonuniform requirements for mechanical performance and the consequence of the growth in volume of the animal. Collagen orientation has therefore been investigated in the cornea,[12,13] heart valve tissue,[14] pericardium,[15] bladder tissue,[16] skin,[17] and aorta.[18]

Crimp, the sinuous arrangement of fiber bundles, has been associated with high strength in tendons[19] as well as in heart valves,[20] with high crimp resulting in high strength. However, in studies of skin (leather) of various strengths, crimp was not observed.[21]

Collagen orientation has been measured by reflection anisotropy,[22] atomic force microscopy,[23] small-angle light scattering,[24] confocal laser scattering,[25] Raman polarization,[26] anisotropic Raman scattering,[27] multiphoton microscopy,[28] and small-angle X-ray scattering (SAXS).[21,29,30]

Our recent study of ovine and bovine leathers of differing strengths[21] found a statistically significant relationship between tear strength and edge-on orientation, and we speculated that this trend may be of a more general nature. We have now measured fibril orientation in seven species of mammals to see if this relationship is found more widely. We used SAXS at a modern synchrotron facility, which allows analysis of a small area (250 × 80 μm), and therefore easy measurement of fibril orientation edge-on in tissues that are of limited thickness;[21] such measurements are difficult to make quantitatively by other methods.

EXPERIMENTAL PROCEDURES

Skins were processed to produce leather by the following procedure. After mechanical removal of adhering fat and flesh, conventional lime sulfide paint, comprising 140 g/L sodium sulfide, 50 g/L hydrated lime, and 23 g/L pre-gelled starch thickener, was applied to the flesh side of the skin at 400 g/m^2. The skin was incubated at 20 °C for 16 h and the keratinaceous material manually removed. The skin was then washed to remove the lime, and the pH was lowered to 8 with ammonium sulfate, followed by the addition of 0.1% (w/v) Tanzyme (a commercial bate enzyme). After 75 min at 35 °C, the treated skin was washed and then pickled (20% w/v sodium chloride and 2% w/v sulfuric acid). Pickled pelts were degreased (4% nonionic surfactant; Tetrapol LTN,

Received: October 31, 2012
Revised: December 23, 2012
Accepted: January 8, 2013
Published: January 8, 2013

dx.doi.org/10.1021/jf3043067 | J. Agric. Food Chem. 2013, 61, 887−892

Shamrock, New Zealand) at 35 °C for 90 min and then washed. The skins were neutralized in 8% NaCl, 1% disodium phthalate solution (40% active; Feliderm DP, Clariant, UK), and 1% formic acid for 10 min. The running solution was then made up to 5% chrome sulfate (Chromosal B, Lanxess, Germany) and processed for 30 min followed by 0.6% magnesium oxide addition, based on the weight of the skins, to fix the chrome, and processed overnight at 40 °C. These wet-blue pelts were neutralized in 1% sodium formate and 0.15% sodium bicarbonate for 1 h and then washed, followed by retanning with 2% synthetic retanning agent (Tanicor PW, Clariant, Germany) and 3% vegetable tanning (mimosa; Tanac, Brazil). Six percent mixed fatliquors were added and the leathers maintained at 50 °C for 45 min, followed by fixing with 0.5% formic acid for 30 min and washing in cold water.

Tear strengths were measured for all samples using standard methods.[31] Samples were cut from the leather at the official sampling position (OSP).[32] The samples were then conditioned at a constant temperature and humidity (20 °C and 65% relative humidity) for 24 h and then tested on an Instron 4467 (Figure 1).

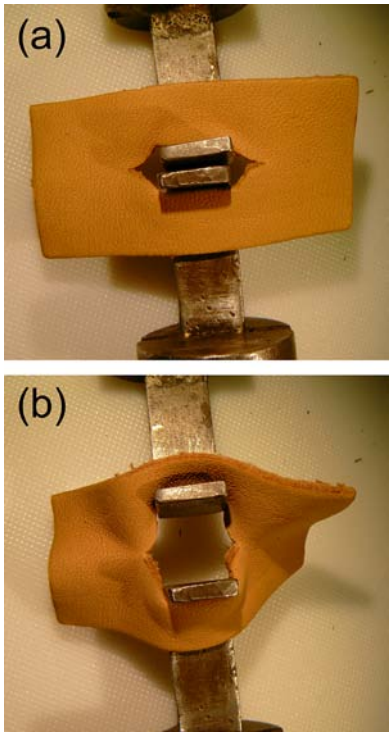

Figure 1. Tear test on a leather sample: (a) at start of test; (b) part way through test.

Samples were prepared for SAXS analysis by cutting strips of leather of 1 × 30 mm from the OSP.[32] Each sample was mounted, without tension, in the X-ray beam to obtain scattering patterns for two orthogonal directions through the leather. For the edge-on analyses measurements were made every 0.25 mm with the samples analyzed from the grain to the corium. For when the beam was directed flat-on (normal to) the surface of the leather, standard samples were cut parallel to the surface, producing a grain sample and a corium sample. These were mounted with the uncut face of the leather directed toward the X-ray beam, and four measurements were made per sample, in a rectangular grid. Diffraction patterns were recorded on the Australian Synchrotron SAXS/WAXS beamline, using a high-intensity undulator source. Energy resolution of 10^{-4} was obtained from a cryocooled Si(111) double-crystal monochromator, and the beam size (fwhm focused at the sample) was 250 × 80 μm, with a total photon flux of about 2×10^{12} ph/s. Diffraction patterns were recorded with an X-ray energy of 8 keV using a Pilatus 1 M detector with an active area of 170 × 170 mm and a sample-to-detector distance of 3371 mm. Exposure time for the diffraction patterns was 1 s, and data processing was carried out using SAXS15ID software.[33]

The orientation index (OI) is defined as (90° − OA)/90°, where OA is the minimum azimuthal angle range, centered at 180°, that contains 50% of the microfibrils.[34,35] OI provides a measure of the spread of microfibril orientation. In the limit, an OI approaching 1 indicates that the microfibrils are parallel to each other and the leather surface, whereas an OI of 0 indicates the microfibrils are randomly oriented. We have calculated the OI from the spread in azimuthal angle of the D-spacing peak, which occurs at around 0.059−0.060 $Å^{-1}$. Each OI value presented here represents the average of 14−36 measurements of one sample. For edge-on mounted samples these measurements were taken at 0.25 mm intervals from the corium to the grain so that the whole thickness of the sample was covered. For flat-on measurements these were taken on a number of points in a grid pattern. For the sheep and cattle samples the averages are derived from 228, 249, and 167 measurements from 15, 14, and 10 samples, respectively, and have been reported previously.[21]

It is not necessary that the samples are highly representative of the particular animal species for general strength−structure relationships to be studied; that there is a range of skins with different strengths is important, although the observed strengths for each species are within industry norms.

The D-spacing was determined for each pattern by taking the central position of several of the collagen peaks, dividing these by the peak order (usually from $n = 5$ to $n = 10$), and averaging the resulting values.

RESULTS

The SAXS patterns display rings representing the collagen fibril repeating structure (Figure 2a). The integrated intensity of the whole pattern enables the position of these peaks to be clearly identified (Figure 2b), and from these the D-spacing is

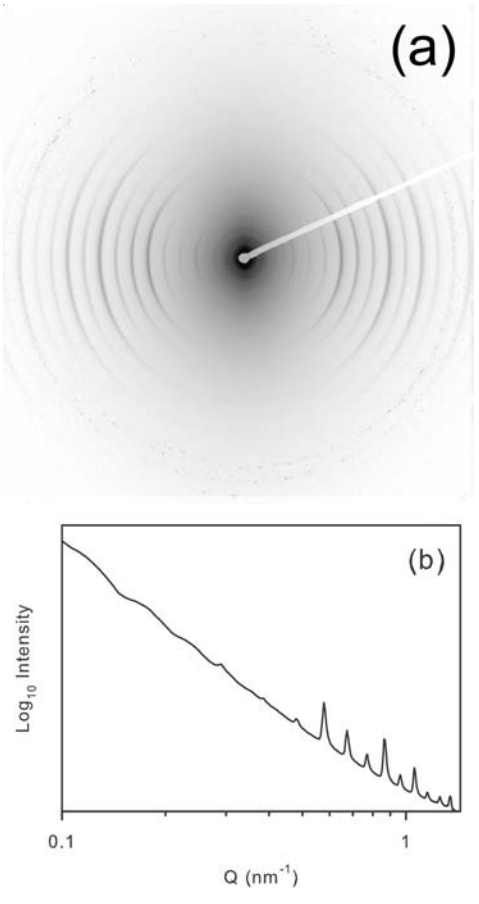

Figure 2. SAXS analysis of leather: (a) raw SAXS pattern; (b) integrated intensity of a whole pattern.

dx.doi.org/10.1021/jf3043067 | J. Agric. Food Chem. 2013, 61, 887−892

determined. The D-spacing varied from 0.628 to 0.653 nm (Figure 3), but there is no significant correlation between D-spacing and strength.

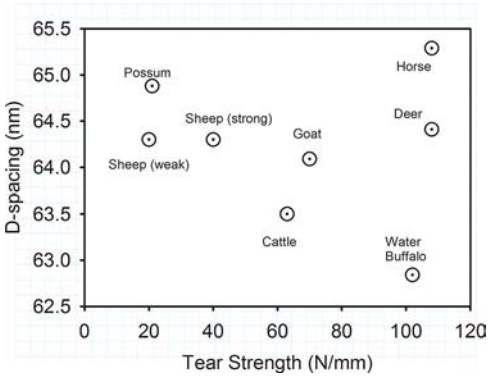

Figure 3. Collagen D-spacing and tear strength for leather from different animals.

For any of the rings visible in the SAXS pattern, which correspond to a peak in the meridional angle, the variation in intensity with azimuthal angle can be plotted (Figure 4), which gives a quantitative measure of fibril orientation, represented here as an OI.

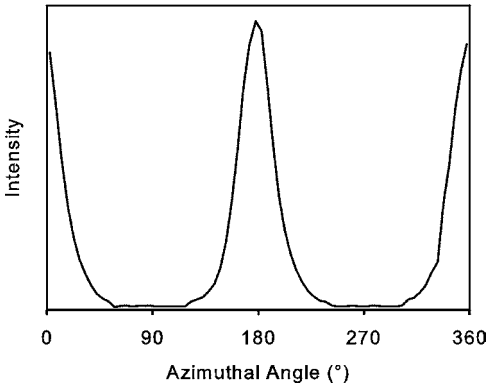

Figure 4. Azimuthal variation in intensity at one value of Q (one collagen peak).

There is a large difference in OI between the measurements taken normal to the leather surface and measurements taken edge-on to the leather. The OI normal to the surface is in the range 0.18−0.35, with the exception of horse leather (Figure 5a), whereas for the edge-on measurements the range is 0.41−0.63 (Figure 5b). Therefore, the major component of fibril alignment is planar.

We find that there is a strong correlation between tear strength and OI (Table 1; Figure 5b) for the edge-on measurements, with a least-squares fitted slope of 0.0024 mm/N ($n = 8$, $r^2 = 0.98$, $P < 0.0001$). This is a remarkably good correlation. Edge-on analysis provides a measure of fibril orientation not frequently accessed. It conveys the degree to which the collagen fibrils are organized in parallel planes as opposed to crossing between the top and bottom surfaces of the skin.

For the measurements on the flat, if we exclude horse leather as an outlier, then there is little correlation between tear strength and OI (Figure 5a) with a least-squares fitted slope of 0.0003 mm/N ($n = 7$, $r^2 = 0.06$, $P = 0.60$), suggesting the slight possibility of a negative correlation. This is the more widely used direction of analysis for collagen fibril orientation measurements.

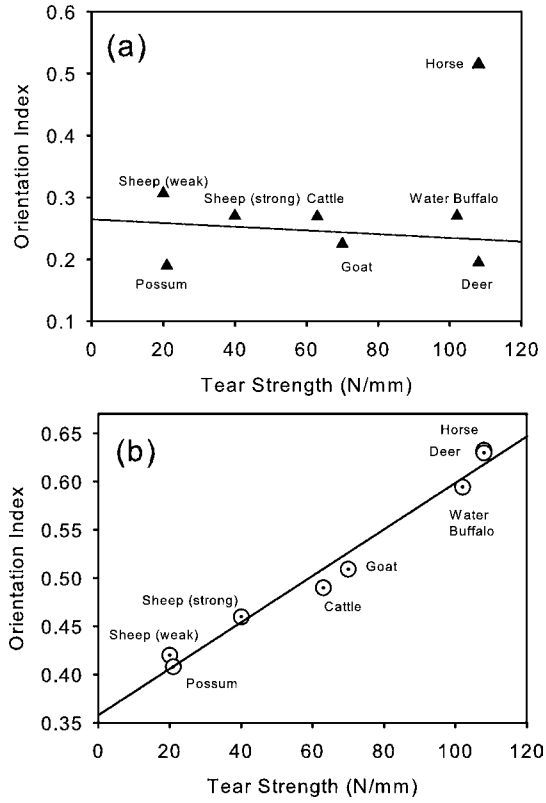

Figure 5. Collagen fibril orientation and tear strength for leather from different animals: (a) measured flat-on; (b) measured edge-on.

Table 1. Leather Tear Strength Compared with Orientation Index (OI) of Collagen Fibrils[a]

animal		tear strength normalized for thickness (N/mm)	OI measured edge-on (average through thickness)
sheep (selected weak)	*Ovis aries*	20	0.420
possum	*Trichosurus vulpecula*	21	0.408
sheep (selected strong)	*Ovis aries*	40	0.460
cattle	*Bos primigenius taurus*	63	0.490
goat	*Capra aegagrus hircus*	70	0.509
water buffalo	*Bubalus bubalis*	102	0.595
deer	*Cervus elaphus*	108	0.630
horse	*Equus ferus caballus*	108	0.633

[a]OI values are the average taken across the thickness of one sample (about 5−10 points) except for sheep and cattle, where these are an average of 6−10 leather samples with 5−10 analysis points for each.

DISCUSSION

D-Spacing. The D-spacing of collagen is known to vary with age,[36] but it has not, to the knowledge of the authors, been linked with mechanical strength. We also do not find a link with strength, therefore supporting the current understanding, although we find a large variation in D-spacing across a large range of strength (Figure 3).

Orientation and Strength. Collagen orientation shows a strong correlation with tear strength when measured edge-on, and this relationship is represented in part by existing models. A relationship between fiber alignment and tensile strength has

dx.doi.org/10.1021/jf3043067 | J. Agric. Food Chem. 2013, 61, 887−892

been modeled previously, where strength is due to the sum of the components of the fibrils that lie in the direction of force in addition to a component due to the other matrix materials.[37] This relationship is represented by eq 1

$$E_z = E_f v_f \int_0^{2\pi} \int_0^{\pi/2} \cos^4 \theta F(\theta, \phi)\, d\theta\, d\phi + (1 - v_f)E_m \quad (1)$$

where E_z is the composite Young's modulus of the material in direction z, E_f and E_m are the Young's moduli of the fibers and matrix, respectively, v_f is the volume fraction of the fibers, and $F(\theta,\phi)$ is the angular distribution function, where θ and ϕ are orthogonal.

This model has been applied to just the measured fibrous collagen, neglecting the contribution from matrix materials, to give an OI, which here we will call OI′ to distinguish it from the differently formulated OI[29,38] (eq 2).

$$OI' = \frac{\int_0^{2\pi} \int_0^{\pi/2} \cos^4 \theta F(\theta, \phi)\, d\theta\, d\phi}{\int_0^{2\pi} \int_0^{\pi/2} F(\theta, \phi)\, d\theta\, d\phi} \quad (2)$$

OI, calculated here from the angle range representing half of the fibrils, can be converted to the integral of $\cos^4\theta$ by numerical methods (where we assume a Gaussian form to the intensity distribution). The OI data plotted for the two orthogonal directions shown in Figure 5 can then be represented as OI′, where, if the model described above is applicable to this system, it should be proportional to the tensile strength. This results in a plot that also correlates with the tear strength data; however, the correlation is poorer than that obtained with the edge-on OI measurements (Figure 6). The fit that includes all data is reasonable ($n = 8$, $r^2 = 0.55$, $P = 0.06$), and this fit improves if the horse data point is removed ($n = 7$, $r^2 = 0.82$, $P = 0.02$). We do not know why horse leather should be an outlier, and this may warrant further investigation. These compare unfavorably with the r^2 of 0.98 for just the OI data edge-on. The reason that this three-dimensional model is a poorer fit than that of the edge-on OI data is considered below.

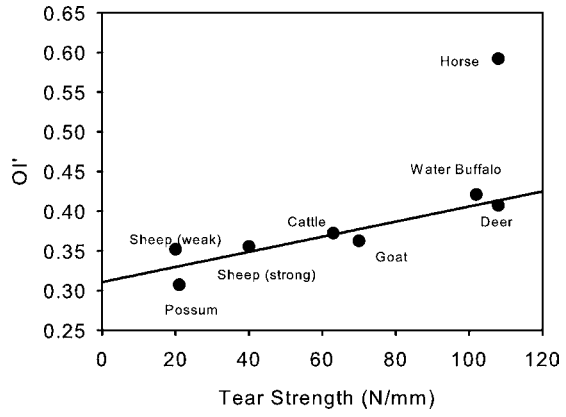

Figure 6. Three-dimensional modeled OI′ based on normalized integral of $\cos^4\theta$.

Alignment and Tear Strength: A More Complex Relationship. Tear strength and tensile strength are related but not identical measures of strength, and it is important to understand the difference between these two measures to be able to relate the model in ref 37 to the tear strength. The basic assumption of the model outlined in ref 37 is that the strength of collagen is along the axis of the fibrils themselves and that the total strength is the simple sum of these fibrils in the direction of tensile force. This is a good model for tensile strength; however, the more useful measure of strength for practical applications of leather is the tear strength, and this does not directly correlate with tensile strength. Tear strength is perhaps analogous to "toughness" in materials.

The ability of leather to resist tear also depends to some extent on the strength perpendicular to the fibril axis, which depends on the strength of the cross-links[11] or the degree of entanglement, and that strength will be less than the strength of the collagen fibrils. However, this appears to be of rather secondary importance compared with fibril alignment. The main component of tear strength for the work presented here is seen to be related to the planar alignment of collagen fibrils. Fibril alignment in the plane has a very strong correlation with tear strength. When the fibrils are not aligned in the plane but instead are perpendicular to the plane (Figure 7a), then any tearing force will need to just separate fibers, pulling in the weakest direction. This arrangement is known as vertical fiber defect and occurs sometimes in Hereford cattle.[38,39] No samples of this type were included in this study.

When the fibrils are rather anisotropic in alignment when measured edge-on (Figure 7b), then the tear strength is likely to be greater than would be found in the vertical fiber defect structure because now there are fibrils running in the direction of the applied force, and maximum strength is obtained when there is a high degree of alignment in this plane (Figure 7c). This trend, depicted in Figure 7 from image a to image c, is what we observe for SAXS measurements over a factor of nearly 5 in strength (Figure 7b), which is a much larger range than has been reported by any other studies.

The reason tear strength does not directly relate to collagen alignment considered in three dimensions (as in eqs 1 and 2) is that tearing is associated with point stresses. To prevent tearing, these point stresses must be resisted. Tearing is used as the industry standard for leather strength because it relates more closely with actual in-service performance than tensile strength. When the tearing process is viewed (Figure 1), looking flat onto

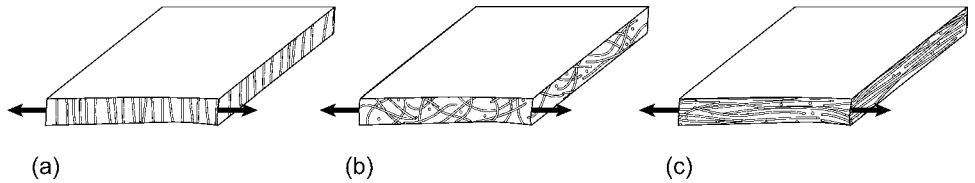

Figure 7. Relationship between collagen orientation index (OI) and strength of skin. OI measured edge-on with orientation that results in leather that is (a) very weak (vertical fiber defect), (b) medium strength (low OI), or (c) strong (high OI). Arrow indicates direction of applied stress in tear measurements.

dx.doi.org/10.1021/jf3043067 | J. Agric. Food Chem. 2013, 61, 887−892

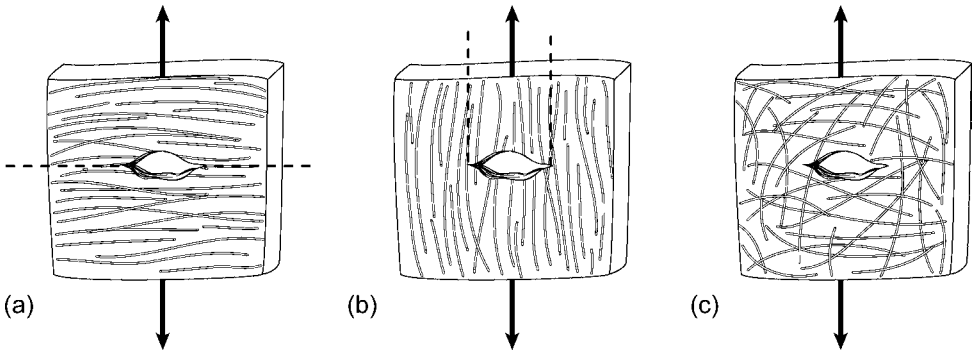

Figure 8. Relationship between collagen orientation index (OI) and strength of skin. OI measured on the flat with orientation that results in leather that is (a) weak (high OI), (b) fairly weak (high OI), (c) strong in all directions. Arrow indicates direction of applied stress in tear measurements. Dashed lines represent probable lines of failure.

the leather, the points where the tearing will occur are at the two ends of the linear cut hole. The fibers that run at right angles to the two edges to the hole (viewed on the flat) resist the tearing process (Figure 8a). However, if all of the fibers run in this direction, then strength may be low due to failure along shear lines (Figure 8b). Therefore, it might be expected that the optimum strength will be associated with a fiber arrangement somewhere between these two extremes, with skin that has a low OI measured in this direction (Figure 8c). Hence, this correlation is one where OI is inversely related to strength, which is what is weakly observed for these leathers (Figure 5a).

Therefore the existing model for strength, where strength depends on the degree of fibril orientation in the direction of stress considering the three-dimensional structure, does not provide an optimal description of the behavior of these materials. It does not take into account the fact that, in practice, a tearing process will follow the weakest part of the structure. The consequence of this is that the direction of the tear front is not well-defined and a degree of anisotropy when viewed flat-on is preferable. The anisotropy, as viewed flat-on, enhances the ability to resist point stresses.

These simplified sketches illustrate the mechanism behind the structure–strength relationship that has been measured for the range of animal skins reported here. What is remarkable is that the relationship between tear strength and edge-on orientation is so quantitative. The strength range across which this relationship holds is much greater than has previously been demonstrated. The correlation also extends across a wide range of mammals.

There is additional information contained in the SAXS patterns that we have not yet analyzed such as the collagen bundle size, which is contained in the low Q region of the pattern. We intend to address this in future work. We are also extending the study to a range of animals from other classes and to other tissue types. We hope, through this work, to build a more complete picture of the structural arrangement of collagen materials and the way in which nature constructs these materials for different applications to provide optimum function. We hope this will lead to an enhanced understanding of the basis of the hierarchical structure of skin and the reasons for the variations between skin from different positions on one animal, between skin of different species, animal classes, and different tissue types. From this study we have found a structural motif that is clearly of primary importance in mammals, but we have not yet demonstrated the generalization of this structural motif to other classes or other tissues.

The work is also being extended to develop an understanding of the changes to the collagen fibrils that take place during the processing of leather. Processing can affect the collagen structure, including both the D-period and the fibril orientation. To understand leather as an industrial material, it is desirable to understand the structural changes that take place as a result of chemical and physical treatments from skin to finished product.

In summary, we have investigated the structure of leather from different mammals to attempt to develop a generalized understanding of structure–strength relationships. We have shown that the tear strength of leather is correlated with collagen fibril orientation parallel to the surface of the leather over a large (factor of 5) range of strengths across seven species of mammals. This has been explained as being due to the strength of the collagen fibrils in their longitudinal axis when suitably arranged to resist the tearing process. This clear demonstration of the structural relationship and consequent insight enables research into other tissues to be better targeted by applying a greater focus to the collagen alignment in plane. We expect that this highly correlated structure–strength relationship extends to tissues other than those studied here.

AUTHOR INFORMATION

Corresponding Author

*E-mail: r.haverkamp@massey.ac.nz.

Funding

This work was supported by the Ministry of Business, Innovation and Employment, New Zealand (Grant LSRX0801). The Australian Synchrotron provided travel funding and accommodation.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was undertaken on the SAXS/WAXS beamline at the Australian Synchrotron, Victoria, Australia. David Cookson and Stephen Mudie at the Australian Synchrotron assisted with data collection and processing. Sue Hallas, of Nelson, assisted with editing the manuscript.

REFERENCES

(1) Fratzl, P. *Collagen: Structure and Mechanics*; SpringerScience +Business Media: New York, 2008; Vol. Collagen: Structure and mechanics.

(2) Floden, E. W.; Malak, S.; Basil-Jones, M. M.; Negron, L.; Fisher, J. N.; Byrne, M.; Lun, S.; Dempsey, S. G.; Haverkamp, R. G.; Anderson, I.;

Ward, B. R.; May, B. C. H. Biophysical characterization of ovine forestomach extracellular matrix biomaterials. *J. Biomed. Mater. Res. B* **2010**, *96B*, 67−75.
(3) Lindeman, J. H. N.; Ashcroft, B. A.; Beenakker, J. W. M.; van Es, M.; Koekkoek, N. B. R.; Prins, F. A.; Tielemans, J. F.; Abdul-Hussien, H.; Bank, R. A.; Oosterkamp, T. H. Distinct defects in collagen microarchitecture underlie vessel-wall failure in advanced abdominal aneurysms and aneurysms in Marfan syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 862−865.
(4) Oxlund, B. S.; Ortoft, G.; Bruel, A.; Danielsen, C. C.; Oxlund, H.; Uldbjerg, N. Cervical collagen and biomechanical strength in non-pregnant women with a history of cervical insufficiency. *Reprod. Biol. Endocrin.* **2010**, *8*, 92.
(5) Narhi, T.; Siitonen, U.; Lehto, L. J.; Hyttinen, M. M.; Arokoski, J. P. A.; Brama, P. A.; Jurvelin, J. S.; Helminen, H. J.; Julkunen, P. Minor influence of lifelong voluntary exercise on composition, structure, and incidence of osteoarthritis in tibial articular cartilage of mice compared with major effects caused by growth, maturation, and aging. *Connect. Tissue Res.* **2011**, *52*, 380−392.
(6) Stok, K.; Oloyede, A. A qualitative analysis of crack propagation in articular cartilage at varying rates of tensile loading. *Connect. Tissue Res.* **2003**, *44*, 109−120.
(7) Zimmermann, E. A.; Schaible, E.; Bale, H.; Barth, H. D.; Tang, S. Y.; Reichert, P.; Busse, B.; Alliston, T.; Ager, J. W.; Ritchie, R. O. Age-related changes in the plasticity and toughness of human cortical bone at multiple length scales. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 14416−14421.
(8) Skedros, J. G.; Dayton, M. R.; Sybrowsky, C. L.; Bloebaum, R. D.; Bachus, K. N. The influence of collagen fiber orientation and other histocompositional characteristics on the mechanical properties of equine cortical bone. *J. Exp. Biol.* **2006**, *209*, 3025−3042.
(9) Jobsis, P. D.; Ashikaga, H.; Wen, H.; Rothstein, E. C.; Horvath, K. A.; McVeigh, E. R.; Balaban, R. S. The visceral pericardium: macromolecular structure and contribution to passive mechanical properties of the left ventricle. *Am. J. Physiol.−Heart C* **2007**, *293*, H3379−H3387.
(10) Commodities and Trade Division, FAO, United Nations. *World Statistical Compendium for Raw Hides and Skins, Leather and Leather Footwear 1990−2009*; Rome, Italy, 2010.
(11) Chan, Y.; Cox, G. M.; Haverkamp, R. G.; Hill, J. M. Mechanical model for a collagen fibril pair in extracellular matrix. *Eur. Biophys. J.* **2009**, *38*, 487−493.
(12) Boote, C.; Kamma-Lorger, C. S.; Hayes, S.; Harris, J.; Burghammer, M.; Hiller, J.; Terrill, N. J.; Meek, K. M. Quantification of collagen organization in the peripheral human cornea at micron-scale resolution. *Biophys. J.* **2011**, *101*, 33−42.
(13) Kamma-Lorger, C. S.; Boote, C.; Hayes, S.; Moger, J.; Burghammer, M.; Knupp, C.; Quantock, A. J.; Sorensen, T.; Di Cola, E.; White, N.; Young, R. D.; Meek, K. M. Collagen and mature elastic fibre organisation as a function of depth in the human cornea and limbus. *J. Struct. Biol.* **2010**, *169*, 424−430.
(14) Sellaro, T. L.; Hildebrand, D.; Lu, Q. J.; Vyavahare, N.; Scott, M.; Sacks, M. S. Effects of collagen fiber orientation on the response of biologically derived soft tissue biomaterials to cyclic loading. *J. Biomed. Mater. Res. B* **2007**, *80A*, 194−205.
(15) Liao, J.; Yang, L.; Grashow, J.; Sacks, M. S. Molecular orientation of collagen in intact planar connective tissues under biaxial stretch. *Acta Biomater.* **2005**, *1*, 45−54.
(16) Gilbert, T. W.; Wognum, S.; Joyce, E. M.; Freytes, D. O.; Sacks, M. S.; Badylak, S. F. Collagen fiber alignment and biaxial mechanical behavior of porcine urinary bladder derived extracellular matrix. *Biomaterials* **2008**, *29*, 4775−4782.
(17) Purslow, P. P.; Wess, T. J.; Hukins, D. W. L. Collagen orientation and molecular spacing during creep and stress-relaxation in soft connective tissues. *J. Exp. Biol.* **1998**, *201*, 135−142.
(18) Gasser, T. C. An irreversible constitutive model for fibrous soft biological tissue: a 3-D microfiber approach with demonstrative application to abdominal aortic aneurysms. *Acta Biomater.* **2011**, *7*, 2457−2466.
(19) Franchi, M.; Trire, A.; Quaranta, M.; Orsini, E.; Ottani, V. Collagen structure of tendon relates to function. *Sci. World J.* **2007**, *7*, 404−420.
(20) Joyce, E. M.; Liao, J.; Schoen, F. J.; Mayer, J. E.; Sacks, M. S. Functional collagen fiber architecture of the pulmonary heart valve cusp. *Ann. Thorac. Surg.* **2009**, *87*, 1240−1249.
(21) Basil-Jones, M. M.; Edmonds, R. L.; Cooper, S. M.; Haverkamp, R. G. Collagen fibril orientation in ovine and bovine leather affects strength: a small angle X-ray scattering (SAXS) study. *J. Agric. Food Chem.* **2011**, *59*, 9972−9979.
(22) Schofield, A. L.; Smith, C. I.; Kearns, V. R.; Martin, D. S.; Farrell, T.; Weightman, P.; Williams, R. L. The use of reflection anisotropy spectroscopy to assess the alignment of collagen. *J. Phys. D: Appl. Phys.* **2011**, 44.
(23) Friedrichs, J.; Taubenberger, A.; Franz, C. M.; Muller, D. J. Cellular remodelling of individual collagen fibrils visualized by time-lapse AFM. *J. Mol. Biol.* **2007**, *372*, 594−607.
(24) Billiar, K. L.; Sacks, M. S. A method to quantify the fiber kinematics of planar tissues under biaxial stretch. *J. Biomech.* **1997**, *30*, 753−756.
(25) Jor, J. W. Y.; Nielsen, P. M. F.; Nash, M. P.; Hunter, P. J. Modelling collagen fibre orientation in porcine skin based upon confocal laser scanning microscopy. *Skin Res. Technol.* **2011**, *17*, 149−159.
(26) Falgayrac, G.; Facq, S.; Leroy, G.; Cortet, B.; Penel, G. New method for Raman investigation of the orientation of collagen fibrils and crystallites in the Haversian system of bone. *Appl. Spectrosc.* **2010**, *64*, 775−780.
(27) Janko, M.; Davydovskaya, P.; Bauer, M.; Zink, A.; Stark, R. W. Anisotropic Raman scattering in collagen bundles. *Opt. Lett.* **2010**, *35*, 2765−2767.
(28) Lilledahl, M. B.; Pierce, D. M.; Ricken, T.; Holzapfel, G. A.; Davies, C. D. Structural analysis of articular cartilage using multiphoton microscopy: input for biomechanical modeling. *IEEE Trans. Med. Imaging* **2011**, *30*, 1635−1648.
(29) Kronick, P. L.; Buechler, P. R. Fiber orientation in calfskin by laser-light scattering or X-ray-diffraction and quantitative relation to mechanical-properties. *J. Am. Leather Chem. Assoc.* **1986**, *81*, 221−230.
(30) Basil-Jones, M. M.; Edmonds, R. L.; Norris, G. E.; Haverkamp, R. G. Collagen fibril alignment and deformation during tensile strain of leather: a SAXS study. *J. Agric. Food Chem.* **2012**, *60*, 1201−1208.
(31) Williams, J. M. V. IULTCS (IUP) test methods − measurement of tear load-double edge tear. *J. Soc. Leather Technol. Chem.* **2000**, *84*, 327−329.
(32) Williams, J. M. V. IULTCS (IUP) test methods − sampling. *J. Soc. Leather Technol. Chem.* **2000**, *84*, 303−309.
(33) Cookson, D.; Kirby, N.; Knott, R.; Lee, M.; Schultz, D. Strategies for data collection and calibration with a pinhole-geometry SAXS instrument on a synchrotron beamline. *J. Synchrotron Radiat.* **2006**, *13*, 440−444.
(34) Sacks, M. S.; Smith, D. B.; Hiester, E. D. A small angle light scattering device for planar connective tissue microstructural analysis. *Ann. Biomed. Eng.* **1997**, *25*, 678−689.
(35) Basil-Jones, M. M.; Edmonds, R. L.; Allsop, T. F.; Cooper, S. M.; Holmes, G.; Norris, G. E.; Cookson, D. J.; Kirby, N.; Haverkamp, R. G. Leather structure determination by small angle X-ray scattering (SAXS): cross sections of ovine and bovine leather. *J. Agric. Food Chem.* **2010**, *58*, 5286−5291.
(36) Scott, J. E.; Orford, C. R.; Hughes, E. W. Proteoglycan-collagen arrangements in developing rat tail tendon − an electron-microscopical and biochemical investigation. *Biochem. J.* **1981**, *195*, 573−584.
(37) Bigi, A.; Ripamonti, A.; Roveri, N.; Jeronimidis, G.; Purslow, P. P. Collagen orientation by X-ray pole figures and mechanical-properties of media carotid wall. *J. Mater. Sci.* **1981**, *16*, 2557−2562.
(38) Kronick, P. L.; Sacks, M. S. Quantification of vertical-fiber defect in cattle hide by small-angle light-scattering. *Connect. Tissue Res.* **1991**, *27*, 1−13.
(39) Amos, G. L. Vertical fibre in relation to the properties of chrome side leather. *J. Soc. Leather Technol. Chem.* **1958**, *42*, 79−90.