The Structure and Performance of Collagen Biomaterials

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Abstract

Type I collagen materials are used in a wide range of industrial applications. Some examples include leather for shoes and upholstery, acellular dermal matrix (ADM) materials for surgical applications, and bovine pericardium for the fabrication of heart valve replacements. The structure of these materials is based on a matrix of collagen fibrils, largely responsible for the physical properties and strength of the materials. How the collagen fibrils themselves contribute to the overall bulk properties of these materials is not fully understood.

The first part of this work investigates a collagen structure defect in leather, known as looseness. Looseness occurs in around 5-10% of bovine leather, and is a result of the collagen fibril layers separating during processing from raw skin to leather. A greater understanding of why looseness develops in leather and a method of detecting looseness early in processing is needed to save tanners a significant amount on wasted processing time and costs. In addition, an environmentally safe method of disposing of defect and waste leather is sort after since the current method of disposing to landfill is causing environmental concern due to the possibility of chromium leaching from leather into the soil as it biodegrades.

Synchrotron based small angle X-ray scattering (SAXS) revealed that loose leather has a more aligned and layered collagen fibril arrangement, meaning there is less fibril overlap, particularly in the grain-corium boundary region. This results in larger gaps in the internal structure of loose leather compared with tight. These gaps could be detected using ultrasonic imaging in partially processed pickle and wet-blue hides as well as leather. Incorporating an ultrasound system into the leather processing line could be a viable method for identifying hides deemed to develop looseness earlier in processing, and these could be diverted down a separate processing line or removed.

Disposing of waste leather by first forming biochar prior to land fill proved to be an effective way of reducing chromium from leaching into the environment. XAS revealed that heating leather to temperatures above 600°C in the absence of oxygen formed a char where chromium was bound in the stable form of chromium carbide. The stability of this structure makes chromium less available to form the toxic hexavalent form in the environment and presents a possible alternative option for environmentally safe disposal of leather.

The second part to this work looks at the correlation between collagen fibril structure in a range of biomaterials in relation to material strength. Leather, ADM and pericardium are three type I collagen based materials which rely on sufficient strength to carry out their

industrial and medical applications. These three materials were studied to try and identify collagen fibril characteristics that relate to high material strength.

SAXS on a range of leather samples from various species revealed that collagen fibril diameter had only a small influence over material strength in bovine leather, and no correlation to strength in leather from other species. Therefore it can be said that the influence of fibril orientation on leather strength takes precedence over that of fibril diameter.

Fibril diameter, d-spacing and orientation were studied in pericardium using SAXS while simultaneously applying strain. It was revealed collagen materials undergo two distinct stages of deformation when strain is applied and incrementally increased. The first stage, at low strain, involves a re-orientation of fibrils to become more aligned. When strain is increased further, the fibrils themselves take up the strain, causing fibrils to stretch and decrease in diameter. The Poisson ratio of the collagen fibrils was calculated to be 2.1 ± 0.7. This high Poisson's ratio indicates the fibrils decrease in diameter at a faster rate than they elongate with strain, and as a result the volume of the fibrils decreases. This feature of collagen could help explain some of the unique behaviours and strength of collagen based materials and could be useful for optimizing industrial applications of collagen materials.

ADM materials, derived from human, porcine and bovine skin was the third collagen material studied. SAXS revealed that each species of ADM material had a slightly different collagen fibril arrangement when viewing the samples perpendicular to the surface. Human ADM was highly isotropic in arrangement, porcine was largely anisotropic, and bovine was somewhere in between the two. Bovine has a more layered fibril arrangement edge on and was the strongest material, followed by human ADM, and porcine was significantly weaker. Bovine was also the most porous material of the three. The discovery of the variations in strength, porosity and fibril arrangement between the three types of ADM materials may help medical professionals select the most suitable material for specific surgical procedures and could lead to a greater number of successful surgeries taking place.

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List of Publications

Published Journal Articles

1. H. R. Kayed, K. H. Sizeland, **H. C. Wells**, N. Kirby, A. Hawley, S. T. Mudie, R. G. Haverkamp. “Age differences in gluteraldehyde cross linking on collagen fibril orientation in bovine pericardium”. *Submitted to Connect Tissue Res* (**2016**).

2. **H. C. Wells**, G. Holmes, J. U-Ser, W. Wei-Ru, N. Kirby, A. Hawley, S. Mudie, R. G. Haverkamp. “A Small Angle X-ray Scattering Study of the Structure and Development of Looseness in Bovine Hides and Leather” **(2016)** *J. Sci. Agri.Food. (Preliminary acceptance).*

3. **H. C. Wells**, G. Holmes, R. G. Haverkamp “Early Detection of Looseness in Bovine Hides using Ultrasonic Imaging” *J. Am. Leather Chem. Assoc.* **(2016)** 111 (3).

4. **H. C. Wells**, K. H. Sizeland, N. Kirby, A. Hawley, S. Mudie, R. G. Haverkamp “Collagen Fibril Structure and Strength in Acellular Dermal Matrix Materials of Bovine, Porcine and Human Origin” **(2015)** *ACS Biomat. Sci. Eng.* 1 (10), 1026-1038.

5. **H. C. Wells,** G. Holmes, R. G. Haverkamp, “Looseness in bovine leather: microstructural characterization” **(2016)** *J. Sci. Food Agric.* 96 (8), 2731-2736.

6. K. H. Sizeland, **H. C. Wells,** G. Norris, R. Edmonds, N. Kirby, A. Hawley, S. Mudie, R. Haverkamp, “Collagen D-spacing and the Effect of Fat Liquor Addition” (**2015)** *J. Am. Leather Chem. Assoc.* **110** (2) 43-53.

7. **H. C. Wells**, K. H. Sizeland, H. R. Kayed, N. Kirby, A. Hawley, S. T. Mudie, R. G. Haverkamp, “Poisson's Ratio of Collagen Fibrils Measured by Small Angle X-ray Scattering of Strained Bovine Pericardium” **(2015)** *J. Appl. Phys.* **117** (4), 044701.

8. **Wells, H. C.**; Sizeland, K. H.; Edmonds, R. L.; Aitkenhead. W.; Kappen, P.; Glover, C.; Johannessen, B.; Haverkamp, R. G. (**2014**). “Stabilizing Chromium from Leather Waste in Biochar.” *ACS Sustainable Chem. Eng.* **2**: 1864-1870.

9. Sizeland, K. H.; **Wells, H. C.**; Higgins, J.; Cunanan, C. M.; Kirby, N.; Hawley, A.; Mudie, S. T.; Haverkamp, R. G. (**2014**). “Age Dependent Differences in Collagen Alignment of Gluteraldehyde Fixed Bovine Pericardium.” *BioMed Res.Int.* vol.2014, Article ID 189197, 10 pages.

10. **Wells, H. C.**; Edmonds, R. L.; Kirby, N.; Hawley, A.; Mudie, S. T.; Haverkamp, R. G. "Collagen Fibril Diameter and Leather Strength." (**2013**) *J. Agric. Food Chem.* **61** (47) ,11524-11531.

Conference Papers, Presentations and Posters

Hannah C. Wells, Katie H. Sizeland, Hanan Kayed, Nigel Kirby, Adrian Hawley, Stephen Mudie, Richard G. Haverkamp, "Poisson Ratio of Collagen Fibrils under Tension." Poster presented at The International Chemical Congress of Pacific Basin Societies, 15-20th December **2015,** Honolulu, Hawaii.

Katie H. Sizeland, **Hannah C. Wells**, John Higgins, Crystal M Cunanan, Nigel Kirby, Adrian Hawley, Stephen Mudie & Richard G. Haverkamp, "Structure and Strength of Neonatal Pericardium for Heart Valve Applications." Poster presented at The International Chemical Congress of Pacifichem Basin Societies, 15-20th December **2015**, Honolulu, Hawaii.

Richard G. Haverkamp, **Hannah C. Wells**, Katie H. Sizeland, Richard L. Edmonds, Nigel Kirby, Adrian Hawley, Stephen Mudie, "Collagen Structure and strength in leather." Conference paper presented at the XXXIII International Congress of IULTCS **2015**, 24-27th November, Novo Hamburgo, Brazil.

H. C. Wells, G. Holmes, R. G. Haverkamp, " Microstructural Characterisation of Looseness in Bovine Leather using Ultrasound." Poster and conference paper presented at the XXXIII International Congress of IULTCS **2015**, 24-27th November, Novo Hamburgo, Brazil.

Hannah C. Wells, Katie H. Sizeland, Nigel Kirby, Adrian Hawley, Stephen Mudie & Richard G. Haverkamp, "A Comparison of Strength and Collagen Structure in Bovine, Porcine and Human Acellular Dermal Matrix Materials for Surgical Applications." Poster presented at the 9th Annual CIGR Section VI International Technical Symposium, 16th – 20th November 2015, Massey University, Albany Campus, Auckland, New Zealand.

K. H. Sizeland, H. R. Kayed, **H. C. Wells**, N. Kirby, A. Hawley, S. Mudie, R. L. Edmonds, R. G. Haverkamp. "Nanostructural Analysis of Bioengineered Tissues for Enhanced Performance." Poster presented at the 9th Annual CIGR Section VI International Technical

Symposium, 16th – 20th November 2015, Massey University, Albany Campus, Auckland, New Zealand.

Hannah C. Wells, Richard G. Haverkamp, "Mechanical Behaviour of Collagen Fibrils with Strain." Poster presented at the Advanced Materials World Congress, 23-26 August, **2015,** Stockholm Sweden. *This poster was chosen for the IAAM Young Scientist of the Year Award for 2015.*

Hannah C. Wells, Geoff Holmes, Richard G. Haverkamp, "Microstructure and Looseness in Bovine Leather." Poster presented at the Advanced Materials World Congress, 23-26 August **2015,** Stockholm, Sweden.

Hannah C. Wells & Richard G. Haverkamp, "An Investigation into Looseness in Bovine Hides and Leather." Symposium presented at the 66th Annual Leather and Shoe Research Association Conference, 24th August **2015,** Queenstown, New Zealand.

Haverkamp, R. G., Sizeland, K. H., **Wells, H. C.,** Kayed, H. R., Edmonds, R. L., Kirby, N., Hawley, A., & Mudie, S. "Strength in Collagen Biomaterials." Poster presented at the Fourth International Conference on Multifunctional, Hybrid and Nanomaterials, 9-13th March **2015**, Sitges, Spain.

Hannah C. Wells, Katie H. Sizeland, Hanan R. Kayed, Nigel Kirby, Adrian Hawley, Stephen T. Mudie, Richard G. Haverkamp, "Poisson Ratio of Collagen Fibrils Measured by SAXS." Poster presented at the Fourth International Conference on Multifunctional, Hybrid and Nanomaterials, 9-13th March **2015**, Sitges, Spain.

Hannah C. Wells, Katie H. Sizeland, Richard L. Edmonds, Nigel Kirby, Adrian Hawley, Stephen Mudie, Richard G. Haverkamp, "Poisson Ratio of Collagen Fibrils." Poster presented at the 1st MBE (Matrix Biology Europe) conference (XXIVth FECTS meeting), 21 - 24 June **2014**, Rotterdam.

Hannah C. Wells, Katie H. Sizeland, Richard L. Edmonds, William Aitkenhead, Peter Kappen, Chris Glover, Bernt Johannessen, Richard G. Haverkamp "Biochar from Leather - the Fate of Chromium." Symposium presented at SLTC 117th Annual Conference 26th April **2014**, Northampton, UK.

Hannah C. Wells, Katie H. Sizeland, Richard L. Edmonds, William Aitkenhead, Peter Kappen, Chris Glover, Bernt Johannessen, Richard G. Haverkamp. "Forming Biochar from Leather Waste to Reduce Leaching of Chromium into the Environment." Symposium presented at 65th Annual Leather and Shoe Research Association Conference, 13th August **2014**, Wellington, New Zealand.

Sizeland, K. H., **Wells, H. C.,** Norris, G. E., Edmonds, R. L., & Haverkamp, R. G. "Collagen D-spacing Modification by Fat Liquor Addition." Symposium Presented at the 65th Annual Leather and Shoe Research Association Conference, Wellington, New Zealand, 13th August **2014.**

Richard G. Haverkamp, Katie H. Sizeland, **Hannah C. Wells**, Hanan Kayed, Melissa M. Basil-Jones, Richard L. Edmonds, Nigel Kirby, Adrian Hawley, Stephen Mudie "Collagen Structure in Useful Biomaterials." Symposium presented at 12th International Conference on Frontiers of Polymers and Advanced Materials Auckland, December 8-13, **2013.**

Hannah C. Wells, Katie H. Sizeland, Richard L. Edmonds, Nigel Kirby, Adrian Hawley, Stephen Mudie, Richard G. Haverkamp, "Collagen Fibril Diameter and Strength." Poster presented at the 12th International Conference on Frontiers of Polymers and Advanced Materials, Auckland, December 8-13, **2013**.

Patent Applications

Hannah C. Wells, Richard G. Haverkamp (2015) "Tissue Analysis Using Ultrasonography" Massey University, NZ Patent application no. 707963

Hannah C. Wells, Richard G. Haverkamp (2015) “Method of Analysing Leather” Massey University, NZ Patent application no. 707978

Client and Technical Reports

K. H. Sizeland, **H. C. Wells**, M. M. Basil-Jones, R. L. Edmonds, R. G. Haverkamp “Leather Nanostructure and Performance” *International Leather Maker* **2014** Sept/Oct p30-34

Richard G. Haverkamp, **Hannah C. Wells**, Katie H. Sizeland “Preliminary Comparison of the Collagen Fibril Structure of Bovine and Porcine Derived Acellular Dermal Matrix Materials” Pilot study report for TEI Biosciences Inc., Boston, USA **(2014).**