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What makes leather stronger? A mechanistic study on the effect of natural/artificial cross-links on tensile strength using small-angle neutron scattering (SANS)

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Introduction

Strength is a very important physical property of leather and determines its range of applications and value as a commercial product. To improve the strength of finished leather aspects such as moisture level, weaving network, fibre coating, space filling and cross-linking chemistry need to be considered during the tanning process.[1] According to the hierarchical structure of collagen-based substrates, it is the precise staggering of triple helical collagen molecules that forms collagen fibril, fibre, and finally, skin and leather.[2, 3] The physical properties of leather have a strong relationship with the intermolecular structure of collagen. During the stretching of leather, fibre bundles will be pulled to a linear shape followed by the spread of tension to the lower level of hierarchical organisation - fibre, fibril and the individual tropocollagen molecules.[4] At the onset point of the strain-stress curve, the cross-links between collagen molecules reach the maximum load and break.[4] Therefore, the intermolecular cross-links play an important role directly in contributing to the maximum stretching force that a single collagen fibril can withstand as well as maintain its molecular packing structure.

In tanned leather, there are two types of cross-links: natural cross-links that exist inherently during the production of collagen fibrils and artificial cross-links introduced during the tanning process. Natural cross-links are a series of tissue-specific polypeptides performing as multi-valent linkages between tropocollagen molecules.[5] Based on their mechanism of formation, the natural cross-links can be classified into immature (reducible on its -C=N- bond) and mature (non-reducible). Histidinohydroxylysinonorleucine (HHL) is a mature natural cross-link found specifically in skin, leading to denser packing of collagen molecules than tendon (without HHL), supported by the less axial periodicity (D-period).[6] Hence, with those natural cross-links holding the intermolecular structure of collagen, a potentially improved tensile strength can be expected. But unfortunately, most of the immature natural cross-links are degraded into corresponding amino acid residues during the early processing stages (liming, bating, pickling, etc.).[7] The instability of immature cross-links results in the loss of many inherent qualities and properties of skin which needs to be reintroduced by virtue of artificial cross-linking (tanning). Thus, the insights into how natural/artificial cross-links affect the tensile strength of leather will suggest a pathway to less intense leather tanning processes, by retaining the inherent cross-links.

To understand the mechanism, structural analysis during stretching of leather samples will be conducted. X-ray and neutron scattering can provide useful information about changes in collagen structure.[8, 9] Previous studies using neutron diffraction on deuterium-labelled crosslinks in collagen with sodium borodeuteride established primary methods on mapping and identifying the cross-linkage sites in collagen fibrils.[10, 11] The positioning is realized based on contrast variation method which allows us to observe specific signals of the labelled species. A similar concept of labelling can also be applied to several types of synthetic tanning agents, such as aldehyde, oxazolidine and carbodiimides. On the other hand, the effect of mineral artificial cross-linkers (tanning agents) on the tensile curves of labelled natural/syntanned leather can also be studied using small-angle neutron scattering (SANS). In this case, the deuterated natural/artificial cross-links work as a gauge for studying the effect of mineral tannages. In summary, in-situ tensile measurements on SANS can provide direct evidence on the contribution of tensile strength by natural cross-links, organic syntans, and possibly, syntan-stabilized natural cross-links for a greener way of producing stronger leather with satisfactory organoleptic properties.

Objectives

The major objective of this project is to investigate the effect of natural cross-links and artificial cross-links on tensile strength and strain-stress profile of collagen fibril, so as to establish and then distinguish the different mechanisms of various types of cross-links involved in the collagen molecular structure in leather. The results will help us devise novel environment-friendly tanning processes producing leather with required physical properties. Detailed objectives are listed below as:

- Quantitate the amount of natural cross-links in fibrillar collagen in untanned/tanned leather samples;

- Label the immature natural cross-links and the syntans;

- Use contrast variation method with real-time mechanical deformation on SANS to establish the mechanisms of different cross-links based on their effects on collagen strain-stress curves.

Methods

Local: This research will be performed at New Zealand Leather and Shoe Research (LASRA) for hide sample preparation and at Australian Nuclear Science and Technology Organisation (ANSTO) for the synchrotron SANS beam, under the advice of Dr. Sujay Prabakar and the beamline scientists.

Source of substrate: Raw, full substance hides will be provided by Tasman Tanning.

Reducing and labelling immature cross-links: Dry skin will be rehydrated with PBS buffer and reduced using sodium borohydride (or sodium borodeuteride, for labelled samples), followed by quenching, washing and lyophilizing.[12]

Cross-link analysis: A well-established LC–MS identification and quantitation method of collagen natural cross-links will be applied for analysing the amount of cross-links in the sampling positions.[7] In summary, the reduced sample will be hydrolysed using 6 M HCl at 105 °C for 24 h, filtered, transferred to a CF-11 column, and eluted with butanol-water-acetic acid (4:1:1, v/v/v) to remove free amino acids. After that, reduced cross-links will be eluted with water, lyophilised for quantitative analysis.

Labelling syntans: The deuteration of commonly used syntans will be performed by the National Deuteration Facility (NDF).

In-situ tensile testing: Material mechanical testing gadgets designed for in-situ tensile testing on wet leather sample at the beamline will be provided by ANSTO.

Expected results

Previous in-situ studies on tensile strength of rabbit skin using small-angle X-ray scattering (SAXS) concluded that curvy collagen fibrils act to enhance skin's resistance to stretching through their rearrangement towards the tensile-loading direction, with rotation, straightening, stretching, and sliding/delamination before fracture. We expect to see the arrangement of the intermolecular natural cross-links and/or the artificial cross-links to change with the increasing external force applied to it, and the profile of such changes are supposed to vary on the characteristics of the cross-links.

Benefit for the local or global leather industry

From this research project, mechanisms which directly contribute to the production of strong leather will be established. The mechanisms can be further applied on the exploration of novel syntans or other tannages with similar cross-linking chemistry to the cross-links known to work by this study. Also, as important as the strength of leather, the project will help us understand the role of mineral tannages on physical properties and so as to find environment-friendly alternatives with equivalent effects.

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