AS TOUGH AS LEATHER: MACRO TO NANO SCALE PERSPECTIVES OF COLLAGEN STABILITY

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Abstract. Leather is a fairly durable and flexible material. Created by tanning animal rawhides and skins, it can be found in many household and personal products. Collagen, one of the major components found in skin, serves an important function in leather—to provide mechanical support by withstanding loads acting on the material. The purpose of this paper is to discuss the basis of the mechanical stability of collagen from macro to nano scale that underpins the functional significance of collagen. There are several types of collagen but the one this paper is interested in are those that participate in higher-order assemblies such as networks, filaments, microfibrils, fibrils, fibres/fascicles. These assemblies collectively form a hierarchical architecture in the tissue from the molecular level to the macroscopic level. The functional significance of collagen is a subject of on-going research as the knowledge gained can direct the development of new technology, e.g. leather design and production. In this paper, the findings related to the mechanical stability of the biological material are highlighted with the help of a recently proposed structure-mechanical framework, underpinning the hierarchical architecture of the collagenous material.

1 Introduction

Biomechanical engineers tend to regard soft connective tissues such as tendons, ligaments and skin as biological examples of fibre reinforced composites comprising collagen fibrous structures embedded in a hydrated proteoglycan-rich extracellular matrix (ECM) [1]. With a remarkable high tensile stiffness and strength, these collagen fibrous structures are responsible for withstanding external loads that act on the tissue [2]. From a fibre composite perspective while the mechanical properties of the tissue are attributed to collagen, it is important to emphasize that the interfibrillar matrix (1) facilitates the load transfer from the hydrated PG-rich matrix (the weak phase) to collagen (the strong phase), (2) minimizes direct contact between fibres by ensuring that the individually fibres are separated, which can in turn prevent a brittle crack from passing completely across a section of the composite, (3) protects the surface of the individual fibres otherwise the fibre surface may experience abrasion by direct sliding contact and this could compromise the mechanical properties.

Animal hides and skins are tough and strong materials. Transforming these raw materials from into a variety of useful as well as desirable products involves a chain of processes. Several processes involve subjecting the collagen in the materials to chemical and mechanical modifications—to treat and soften the hides—while minimising possible damage to the properties of toughness and strength of the hide. There is also a need to design efficient methods that are environmentally sustainable for processing leather. At the tannery, often a significant amount of water, as well as chemicals which are toxic and environmentally undesirable, is used, but the carbon footprint is further enlarged as energy is also required to drive these chemical reactions [3].

Collagen molecule is composed of three polypeptide chains exhibiting a triple-helical structure, and the molecule is often referred to as tropocollagen molecule. How the tripeptides contribute to the stability of the collagen molecule has been a subject of great interest in the 70's from the perspective of fundamental research [4]. As pointed out by Professor Eckhart Heidemann, among the three areas of research in leather science, namely technique application, product/process development and fundamental research, the last is recognised as the necessary basis for the discovery of new products especially where it can produce relevant new insights in relation to the practice [5]. Professor John Ramshaw has addressed an up-to-date landscape of the key areas of the biochemistry and structural biology of collagen in a previous Heidemann lecture. As a continuation of this subject, this paper discusses recent findings on the role of collagen in regulating the mechanical stability of the biological material as follows

(1) Physicochemical factors affecting collagen stability;
(2) Mechanics of collagen: stability and cross-links;
(3) Hierarchical architecture of collagen.

The recent findings are important because they have been carried out using new technology and more accurate methodology. This is expected to encourage further development of leather product to optimize for collagen stabilization and achieve a more sustainable future for the leather industry.

2 Physical and chemical factors affecting collagen stability

2.1 Overview

This section briefly highlights the similarities and differences between skin and leather in order to lend support to biomechanics-related arguments for establishing a simple picture of the mechanical stability in the leather. With regard to mechanical stability, the subjects of discussion are leather processing, and agents of deterioration namely heat and mechanical wear. With regard to heat, the discussion on deterioration effects is complemented by highlights on some recent findings on tanning process as a protection against heat. With regard to mechanical wear, the discussion is on structural changes and corresponding mechanical changes when leather is in service, complemented by differentiating these effects from mechanical treatment to leather during processing. Obviously deterioration due to heat and mechanical wear are two of the many factors; the other factors are oxidation, metals and salts, and water.

2.2 Extracellular matrix of skin versus leather

As you probably do not need reminding, collagen makes up the bulk of ECM of skin (as well as tendons and ligaments), amounting to about 70–80% of the dry weight of the skin [6, 7]. So how different is leather collagen structure from skin? The processing of skin leading to leather-the common stages being fresh green, salted, pickled, pretanned, wet blue, retanned, dry crust, dry crust staked[8]-removes many ECM components, e.g. epidermal cells, proteoglycans, elastin, but collagen appears not to be dramatically affected by the processing, even after liming, bating, and pickling are applied [9]. (NB: Elastin, an ECM component in skin well-known for providing the elastic properties of skin, may not have a significant effect on the leather mechanical properties as elastin degradation, e.g. elastin removal by elastase, in leather did not lead to a significant change in the stiffness, tensile strength and extensibility [10].) The collagen fibrils now become connected by synthetic chemical bonds as well as natural chemical bonds. These bonds may enhance the yield strength of the leather because the fibrils may be unable to slide pass one another easily. However, these bonds may also undesirably stiffen the material; thus glycerol is introduced into the interfibrillar matrix during fat liquoring (Liu, 2003). The final product leads to two distinct layers in

the leather which are vaguely related to the dermis of skin: a fine densely packed fibrous layer and coarser layer which we refer to as the grain and corium layers respectively. Much of the organisation of collagen found in skin is retained in these layers; in skin the fibres (and fibrils) are oriented according to the Langer lines [11]. The other features of collagen fibrous structures in the skin namely high slenderness and characteristic light-dark bands referred to as the D-periods on the structure [6], are also retained in leather. Knowledge on the differences and similarities between skin and leather are important as it will enable us to know how structure of skin influence the properties and performance of leather.

2.3 Chemical processes

The general mechanical response of connective tissue when subjected to an external load follows the stress-strain profile that is depicted in Figure 1. As pointed out in previous section, there are 8 main stages of processing leather from skin, namely fresh green, salted, pickled, pretanned, wet blue, retanned, dry crust, dry crust staked. How do the stress-strain profiles look like for leather during the different stages of processing? It turns out that the stress-strain curve varies dramatically with the respective stages as reported by Sizeland and co-workers (Sizeland et al., 2015). Note that the analysis is not entirely complete as the authors did not carry out test on skin-related specimen, so it is not possible to know how much of the mechanical behaviour of skin has changed after leather processing.

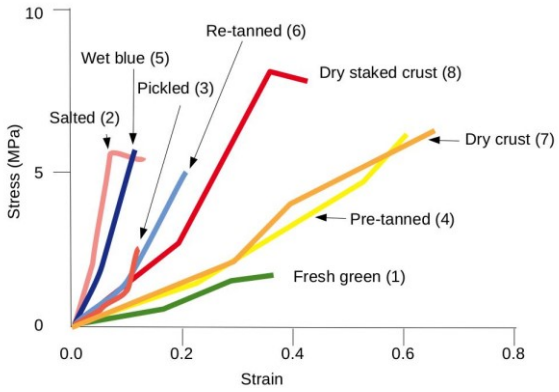

Fig. 1. Sketch of stress versus strain curves of leather material at different processing stages (labelled 1-8).Thereafter, all references to these stages in the main text are labelled as #1 (fresh green), #2 (salted), #3 (pickled), #4 (pre-tanned), #5 (wet blue), #6 (re-tanned), #7 (dry crust), #8 (dry staked crust). The stress-strain curves were derived from ovine skin. The curves were intended for a very general comparison of the shapes because the tests were carried out with no specific considerations for the orientation of the material to account for collagen fibril direction; an absolute comparison of the magnitudes of the stresses and strain is not possible [8]. Reprinted (adapted) with permission from Sizeland KH, Edmonds RL, Basil-Jones MM, Kirby N, Hawley A, Mudie S, Haverkamp RG: Changes to Collagen Structure during Leather Processing. Journal of Agricultural and Food Chemistry 2015, 63(9):2499-250. Copyright (2015) American Chemical Society

Nevertheless, it is easy to point out the similarities with regard to the features, namely the existence of a toe region, non-linear (attributing to elasticity-related mechanisms) region in the low stress region, point of inflexion (attributing to failure mechanisms, such as yielding, leading to plasticity) at higher stress region and abrupt reduction in stress as the material breaks apart, beyond the maximum stress point. These features are reflected in skin [12] as well as ligaments [13] and tendons [14]. The more interesting observations are the effects from the various processing stages as reflected in the extents of the stress and strain, as well as the stiffness at low stress regions (Fig. 8). The different effects arising from the variety of processes underpin the collagen mechanical

stability at different hierarchical levels (section 0). Unfortunately, current findings of how the stress-strain behaviour leather changes during processing, by attributing to the collagen fibril alignment (fibrillar level) [8] and collagen D-spacing (molecular level) [8], does not lend to a complete understanding as the effects at the other hierarchical levels in leather are still not clear. These are important considerations for further study especially where newer findings, such as new fat liquoring agents [15], and recommendations to lower the amount of chromium use in stabilizing collagen [16], are recently proposed.

2.4 Thermal degradation

How does high temperature affect the mechanical stability of leather? There are many studies on high temperature effects, covering hydrothermal effects and shrinkage temperature {Kite, 2006 #5606}. Here, this section focuses on changes at the molecular level, addressing how the collagen molecular structure is affected. The basic mechanics at elevated temperature, e.g. at 120 °C, involves alteration (mainly shortening) to the D-period, as seen in dry collagen [17], possibly contributed by the shortening of the pitch of the collagen helix but predominated by axial sliding of molecules [17]. further shortening of the D period occurs [17]. The reversibility of the effects depends on the temperature: low temperatures produce effects (attributing to molecular unfolding occurring locally, i.e. possibly resulting from a small proportion of hydrogen bond ruptures) which are reversible [18]; high temperatures produce effects (the molecule unfolds into a random (coiled) structure) which are irreversible [18]. Elevated temperature effects appear to be time-dependent: the longer the duration in which it is subjected to elevated temperature, the higher the degree of randomness in the structure [18]. The mechanics of unfolding points to the disruption to the hydrogen bonds that binds the helices [18]. At the macroscopic level, the molecular changes may not affect the bulk mechanical properties appreciably at short heating duration [19]; interestingly, Weadock has reported that long duration results in increase extensibility, fracture strength and stiffness of the tissue [20].

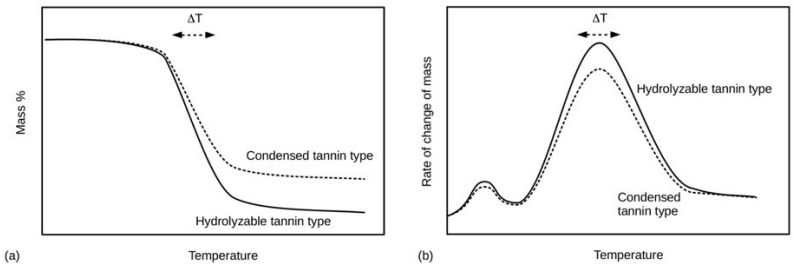

Fig. 2. Sketch of graphs of (a) mass loss and (b) rate of change of mass versus temperature to illustrate how tannin type affects the thermal stability of leather. Here ΔT represents a range of temperatures. For instance, in a previous study [21], ΔT = 300-400 °C (for the details of the experimental findings see Sebestyen et al. [21]).

Tanning is intended to stabilise the collagen molecule by creating a more permanent bonding between the helices [21, 22]. The degree of stability depends on the tannin molecule with condensed tannin type being more superior than hydrolysed tannin type; the former yields lower mass loss (TGA curves) and smaller rate of mass loss (DSC curves) over a range of high temperatures [21] (**Fig. 2**). Currently findings of what really happened during heating by attributing to collagen molecular level effects, i.e. mechanics of bonding, does not lend to a complete understanding as the effects at the other hierarchical levels in leather are still not clear.

2.5 Mechanical wear and tear

How mechanical loading affects the mechanical stability of collagenous material such as leather has been dealt with in studies on (i) leather processing and (i) leather during service.

Repeated loading that form part of leather processing namely milling and staking is intended to yield the desired softness in leather [23]. Milling is likened to a preconditioning process to ensure consistency of results, borrowing from biomechanical testing of soft tissue [24], prior to the leather being deployed for use. At the macroscopic level, analysis of the hysteresis curve revealed that a milled leather results in smaller energy loss than unmilled leather [23]. As the number of cycles increases, the strength increases but the strain at fracture decreases [25] (**Fig. 3** b). Clearly there is an optimal level to achieve high strength without compromising too much on reduction in the extensibility. At the fibrillar level, milling ensure that collagen fibrils are recruited into the desired orientation. At the tropocollagen molecular level, the cyclic stress experiences by a dry leather may minimise hydrogen bonding within the fibrils.

Static stretching of leather material, along its long axis (parallel to the backbone), to a desired length and maintaining the leather in this state over time has been proposed as part of a leather processing stage (namely during the wet blue stage) intended to achieve maximal area [26], which is important for optimising the profit, with consequential enhancement to collagen mechanical stability. At the macroscopic level, stretched leather stiffness is dramatically higher (particularly those treated to low angles with respect to the long axis) than those without pre-strain; the stiffness increase also depend on the amount of stretch applied during the pre-strain treatment [26]. At the fibrillar level, this is explained by the result of fibrils oriented predominantly along the pre-strain axis.

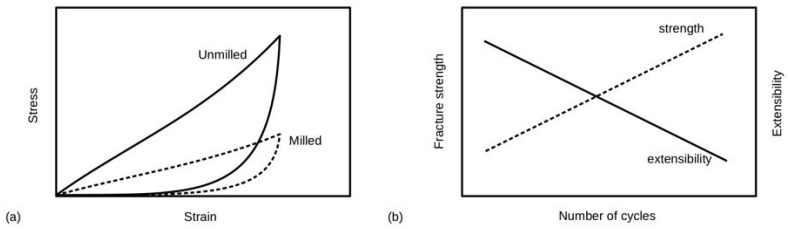

Fig. 3. Sketch of graphs of (a) stress versus strain (the stress is of the order of 10^6 Pa as reported elsewhere [23]) and (b) fracture strength versus number of loading cycling.

Tearing could occur when the leather is in service. In the latest attempts to understand the mechanical stability of collagenous materials, like leather, to resist tearing, Kelly and co-workers found that there was an appreciable difference in the tear strengths (whether torn in parallel or perpendicular to the backbone) in leather (tanned) processed without strain and leather prepared by tanning under strain; the tear strength increases in both direction [27]. This finding is important because it correlates to orientation changes at the fibrillar level whereby a greater degree of alignment was observed with pre-strained leather compared with unstretched leather [27]. Of note, it has been pointed out that tannins may help to increase wear resistance. This is because hydrolysis of tannins, which can occur within the leather and is not desirable from the point of view as a loss of tanning material, results in carboxylic acid moieties by-products which is deposited in the tanning pits and this may contribute to the water resistance and wear properties of the leather [28].

3 Collagen mechanics in relation to stability and cross-links

3.1 Overview

This section is intended to highlight how the interfibrillar matrix, collagen volume fraction, fibril orientation as well as other individual fibril characteristics, and, finally, interfibrillar cross-links influence the mechanical stability of collagen.

3.2 Interfibrillar matrix

The interfibrillar matrix in ECM of tendons, ligaments, dermal skin may be regarded as a hydrated proteoglycan-rich ground substance which serves to hold the collagen fibrils together. Specifically, the interfibrillar matrix (1) protects the collagen fibrils from mechanical damage, (2) binds the fibrils and (3) provides a medium for load transfer from the interfibrillar matrix to the collagen fibrils [1, 2, 29]. Thus it is important to be able to measure the mechanical properties of the matrix to investigate the extent of some, if not all, of these assumptions.

A useful model for understanding the interfibrillar matrix is the rule-of mixture, complemented by the shear-lag model. Let E_{CT} and E_{cf} be the moduli of elasticity of the tissue and collagen fibril, G_m be the shear modulus of the interfibrillar matrix, V_f and V_m be the volume fractions of collagen and the interfibrillar matrix, L_f be the fibrillar length, and A_f, r_f and R be the fibrillar cross-sectional area, fibrillar radius and inter-fibrillar distance, respectively. To order-of-magnitude, according to the rule-of-mixtures for stiffness, estimates of the interfibrillar matrix stiffness (E_m) may be derived from the mathematical model,

$$E_{CT} = E_{cf}V_{cf}a + E_mV_m, \quad (1)$$

where the coefficients,

$$a = [1-\tanh(bL_f)/[bL_f]], \quad (2)$$

is derived from the Cox shear-lag model [30], and

$$b = \sqrt{([G_m/E_{cf}][2\pi/A_f]/\log_e(R/r_f))}, \quad (3)$$

is an important parameter that is used to describe the effective length of the fibril, $L_f' = bL_f$ [31].

Of note, the larger the value of G_m/E_{cf} the more rapidly the stress in the fibril increases with distance from the fibril end and consequently, higher E_{CT}.

Moisture absorption by leather material confers flexibility to the material. In connective tissues such as tendons, deformation at the interfibrillar matrix level is correlated to the deformation at tissue level. [32-35]. Deformation at the interfibrillar matrix is regulated by interfibrillar shearing (by shear-lag mechanism or even shear-sliding mechanism); this involves transferring stress from the matrix to collagen fibrils [33, 34]. Since the interfibrillar matrix of tendon is highly hydrated, it suggests that water plays an important role in the flexibility of the tissue by provides a lubricating effect in collagen fibrils. A similar conclusion is recently established for leather: Kelly and co-workers further suggested that moisture absorption could result in a larger lateral spacing between collagen molecules in fibrils [36]. This conclusion should provide important consideration for leather processing, because it is known that salting (#2) causes dehydration to some extent.

But what is the nature of the interfibrillar matrix? To address this question we note that the various reactions occurring during the processing of leather would breakdown and remove a lot of the native interfibrillar matrix components, such as proteoglycans, and replaced by chemicals used in leather processing. For instance, glycosaminoglycans and other components are removed during the pickling stage (#3) [8]. Since some, not all, of these non-fibrous ECM protein molecules that were depleted from the ECM could be responsible for regulating the interfibrillar shear mechanics

and the stress transfer mechanisms [8], one would expect to introduce a compensatory approach to restore the material properties. This is covered in two ways: tannins and fat liquoring (tanning stage #6, the two components are introduced to soften the material), collagen cross links (pretanned stage #4, where collagen fibrils are cross-linked) [8], as well as water (when in service) and fats/oils and waxes (after tannage) [37]. At normal environmental conditions, water present in the leather may be categorised into two groups: multilayer water and molecularly bound water[37, 38]. The former, which is regarded also as free water, is believed (1) to be localized within the interfibrillar space in the form of multilayers; (2) to exist bound to proteins as monolayer layer; (3) to be held by mixture of strong and weak bonds (such as hydrogen and van Der Waals); (4) to be make up of about 15% volume fraction, at a relative humidity of 65% [37]. It is believed that loss of the free water may result in leather stiffness but this is reversible as long as the collagen structure is not significantly damaged during loading [37]. The latter, i.e. bound water, is present in the collagen in such as way that it is not available for dissolving electrolytes [37]. Thus, it is the multilayer water, tannins and other chemicals (i.e. present due to fabrication and use) in the interfibrillar matrix which may be regarded as substitutes for the original interfibrillar matrix, to facilitate the interfibrillar shear (i.e. shear-lag and shear-sliding, as described by eq.s (4) and (5), respectively in the following section). Deterioration of the interfibrillar matrix may occur when the leather is in service, e.g. attacked of the tanning agents by radical mechanisms during photochemical reactions [39]. This may compromise collagen stability: in the presence of a degraded interfibrillar matrix, when leather is subjected to external loads, high frictional stress could be generated between the fibrils as the fibrils attempt to slide pass one another.

3.3 Collagen

3.3.1 Collagen volume fraction

Collagen volume fraction, V_f, is a measure of the total volume of collagen in the tissue specimen with respect to the total volume of the tissue. V_f is an important parameter as it modulates the stiffness and strength of the tissue according to the rule of mixture for the respective mechanical properties. V_f could change with age and age-related changes as such could result in changes in the mechanical properties; in age-varying tendons, the strength and stiffness increase linearly with V_f [40]. These changes may be indirectly affected by sex hormones, such as estrogen and androgen, e.g. in the tissue of skin and gingival fibroblasts [41], which are responsible for (i) regulating proteoglycan turnover (which in turn may influence the fibril size) and (ii) hydroxyproline content; the latter is probably more important because it is a major component of collagen and any changes would directly influence collagen stability [41]. Indications of changes in V_f may be observed in changes in the fibril-bundle packing [41], fibril packing [41, 42], state of hydration [43] and Poisson's ratio of the collagenous material undergoing deformation under external load [44]. With regard to the collagen fibril-bundle, in young individuals, the bundles are tightly packed from the papillary to the deep dermis. With age, the bundle thickness increases and bundle packing density decreases in the dermis [41]; there is no appreciable change in the thickness of the bundles in the papillary dermis [41]. It is important to note the underpinning arguments for the changes in V_f in the physical feature (i.e. fibril packing) processes (state of hydration, and deformation mechanics parameterized by Poisson's ratio) arise from different causes: (1) the changes in fibril packing within bundles underpins changes in fibril-fibril interactions [42]; (2) the hydration process underpins the spacing between fibrils, as well as the gaps between the tropocollagen molecules, depending on how much water is expelled [43]; (3) the Poisson effect underpins the extent of cross-links between the collagen molecules in the lateral and longitudinal direction of the molecules [44].

3.3.2 Collagen fibril network

The network of collagen fibrils in dermal tissue features a somewhat randomly aligned state. However, when the tissue is deformed under a tensile load, the entire network can be recruited in tension by realigning the fibrils through a significantly large angular displacement, e.g. 50 degree, in the direction of the applied load [45]. Collagen is mechanically stabilised in this way for as long as the load does not exceed the fracture strength. Since the degree of fibril orientation determines the tear strength of the leather [27], high tensile strength may be achieved for the finished product which possessed highly aligned fibrils (if the strength is measured in the stretched direction of the load) [46]. The tensile strength depends on the direction of loading on the leather material in leather with highly aligned fibrils (**Fig. 4**a). The tensile strength, as well as stiffness, decreases as the angle of loading (with respect to the backbone) increases [26, 47],.

However, finished products of leather with highly aligned fibrils may not be desirable, especially if looseness occurs, a defect which (can be detected during quality control) decreases the product value because it does not make the leather look good. Some parts of a hide, namely shoulders and flanks, can give rise to looseness [46]. The highly aligned fibrils in loose leather occur throughout the thickness of leather, compared to tight leather [46]; loose leather is also found to have less densely packed fibrils, particularly in the lower grain region [46]. While the tear strength in the direction parallel to the aligned fibrils is high, the tear strength in the direction perpendicular to the aligned fibrils is low [27]. It is found that the looseness manifests during leather processing and exacerbates at different stages [8]. In particular, the fibril alignment is shown to develop during the wet blue stage (#5) [48]; the degree of fibril alignment follows a trending increase as the material undergoes different stages of processing. Unfortunately, what exactly causes looseness is still not clear.

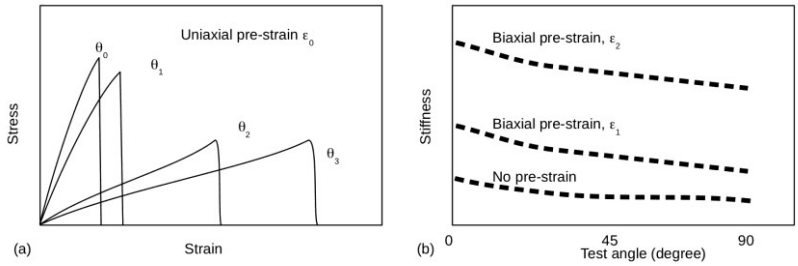

Fig. 4. Mechanical response of leather. (a) Sketch of stress-strain curves of leather treated to drying with a prestrain ε_0, uniaxially tensile tested to rupture, stretched along long axis, i.e. parallel to the backbone (for instance, ε_0=0.3 [26]). The angles are defined with respect to the backbone, θ_0, θ_1,θ_2 and θ_3. The angles reported in a previous study were 0°, 15°, 60° and 90°, respectively; the stress was of the order of 10^6 Pa (MPa) [26]. (Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Journal of Materials Science, Boote C, Sturrock E J, Attenburrow G E, Meek K M, Psuedo-affine behaviour of collagen fibres during the uniaxial deformation of leather, **37** , 3651–3656 [COPYRIGHT] (2002).) (b) Sketch of stiffness of leather, treated to drying under varying pre-strains at different loading angles, with respect to the backbone. In a previous report [47], the pre-strains applied, ε_1 and ε_2 , corresponded to 0.1 and 0.2, respectively; the stiffness was of the order of 10^6 Pa (MPa). (Adapted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Journal of Materials Science, Sturrock E J, Boote C, Attenburrow G E, Meek K M, The effects of the biaxial stretching of leather on fibre orientation and tensile modulus, **39** , 2481-2486 [COPYRIGHT] (2004))

Several factors can affect the alignment of collagen fibrils, namely hydration, leather thickness and pre-strain treatment. The fibrils in dehydrated leather materials are less aligned than hydrated ones [8]. Leather thickness also affects the fibril alignment in that the fibril orientation in the grain layer is vastly different from that in the corium layer [49]. The amount of pre-strain influences the stiffness; stiffness increases with increasing pre-strain values dramatically (Fig. 4b). It should be emphasized that changes in fibril alignment is not merely a 2D planar effect; a proportion of the

realignment of the fibrils also comes from the planes perpendicular to the surface of the leather during pre-strain and it is proposed that this proportion could also contribute to the increase stiffness [47].

3.3.3 Collagen fibril diameter

The mechanical stability of collagen at the fibrillar level addresses the stress uptake in the fibril during loading, described by

$$\sigma_{cf}(z)= \varepsilon_{CT}E_{cf}[1-\cosh(bL_f z)/\cosh(bL_f)], \quad (4)$$

for the case of elastic stress transfer, which is associated with initial loading [2, 50]. Here b is given by Eq. (3). The mechanics of stress uptake changes during plastic stress transfer, which is associated with latter stages of (post-yield) loading, and this is described by

$$\sigma_{cf}(z)= 2\tau[L_f/r_f](1-z) \quad (5)$$

[1, 2, 51]. In all models, the cross-sectional size (e.g. radius (r_f) or diameter ($D_f=2r_f$)) of the fibrils plays an important role in determining the stress uptake. Thus, understanding the size distribution and how radius changes could provide insight into how how the fibril takes up stress.

The tear strength of some types of leather, e.g. bovine origin, is sensitive to D_f. The larger the D_f the higher the tear strength [52]. However, in other types, i.e. ovine, the tear strength is independent of D_f, suggesting that other factors could have confounded this relationship, e.g. τ, E_{cf}, G_m and L_f [53]. More research is needed to illuminate how each of these factors contribute to the overall strength of the leather.

Type V collagen may be important for regulating the initiation of collagen fibril assembly [54]. If this initiation were to be disrupted, e.g. when the type V collagen content is low, this may result in a mixture of two morphologically different fibrils, namely a population of fibrils (normal) cylinder-like cross-section and a population of fibrils with abnormally large irregular cross-section [54]; less fibrils are also formed and this results in lower collagen content [54]. Although bimodal distribution of D_f is found in young to old individuals, it is observed that with age, the cross section profile of the population with larger D_f appears to be more irregular[40]. While the resilience of the tissue increases with fibril diameter for both populations of fibril associated with small and large diameters only the fracture toughness of the tissue increases with fibril diameter for the large-diameter population (and the opposite effect occurs with the small-diameter population) [55].

Hides and skins containing defects such as lesions caused by demodex bovis mites-appearing as ragged fibrous cavities which are hard and unsightly [56]-are disposed leading to financial losses to farmers, traders and the tanning industry [57]. Abu-Samra and co-worker remarked that the fibres around the cavities were distorted, thinner (meaning, D_f is smaller) than normal [58]. They reported quantitative results showing that the tensile strength, tearing load, percentage elongation were significantly lower than non-infected ones [58]. However, no quantitative and qualitative evidence of the profile of the fibres (or fibrils) was shown; it was not possible to gain further insight about how the abnormal structure of the fibres (or fibrils) affect the mechanical properties of the leather material.

3.3.4 Fibril-matrix interface

When collagenous tissues deform under an external load, the deforming hydrated PG-rich ground substance shears on the collagen fibrils; the natural cross links between fibrils and between fibril-matrix are deformed and this generates an interfacial shear stress. Consequently, shear on the fibril causes the fibril to stretch and generate stresses to resist the external load that is attempting to pull the tissue apart. Fig. 5 a and b shows the profile of the shear stress as a function of distance

along the fibril from the centre (Z=0) to the end (Z=Lf/2) during elastic stress transfer (which corresponds to an elastically deforming tissue) [29] and during plastic stress transfer (tissue post-yield stage) [1, 2, 59]. Consequently, the non-linear profile in the former leads to a non-linear axial stress distribution described by Eq. (4) while the uniform stress profile in the latter leads to a simple linear axial distribution described by Eq. (5).

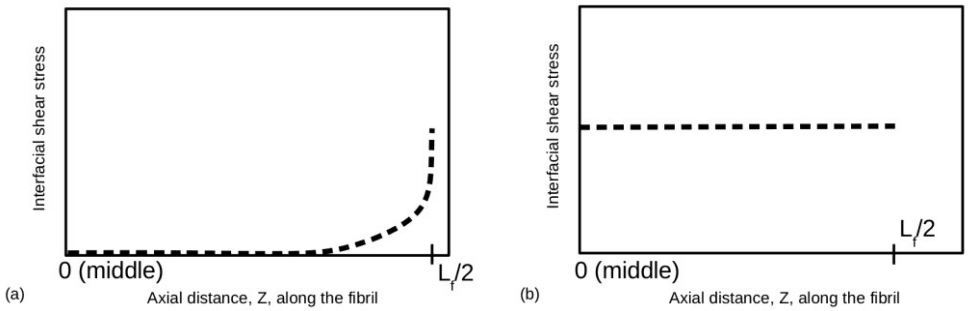

Fig. 5. Graphs of interfacial shear stress versus axial distance along the fibril during (a) elastic stress transfer, and (b) plastic stress transfer stages. The graph shows the stress level from the middle (Z=0) to the end ($Z=L_f/2$) of the fibril.

With regard to leather, during leather processing, various components of the hydrated proteogylcan-rich ground substance in ECM are removed (for instance, glycosaminoglycans (GAGs) are removed by liming and bating), and the intermediate product at the pickled stage (#3), would have fewer natural cross-links [8]. However, new cross-links are created during the pretanning (#4) and wet blue (#5) stages [8]. It was observed (**Fig. 6** a) that the D-period of collagen during the pickled stage was much higher than that during the wet-blue stage, suggesting that the collagen fibrils were appreciably stretched in the former as compared to the latter [8]; this could compromise the collagen stability as shown by the small strain range in the former as compared to the latter. Nevertheless, the presence of new cross links in latter stages up to the finished product suggest that these shear stress response described in **Fig. 5** a and b are expected to be applicable to the fibril-matrix interface in leather material. **Fig. 6** b shows the results of an attempt to investigate whether GAGs are implicated in the cross-linking of fibrils to the matrix and between fibrils. With respect to native tissue, the absence of GAGs (removed by Chondroitinase ABC) showed no appreciable change to the orientation index. GAGs have been the 'usual suspect' for natural cross-links for quite a long time. These natural cross-links at the fibril-matrix interface facilitate the stress transfer within ECM, based on observation of micrographs of GAG side chain, associated with proteoglycans (PGs) bound on collagen fibrils, such as decorin PG-a member of the family of small leucine-rich PGs (SLRPs) [60-71].

But in the last 10 years or so, investigations to study how tissue mechanical properties are compromised by removing GAGs (by Chondroitinase ABC) has yielded negative results [24]. Although the exact nature of the natural cross links are not yet known, **Fig. 6** b shows that introducing artificial crosslinks (using glutaraldehyde) into the collagenous tissue can result in a significant effect on the orientation index when the tissues are stretched, implicating that the artificial crosslinks actually works to anchor between fibrils, and to realign the fibrils in the direction of the applied load. Glutaraldehyde is a tanning agent that functions as collagen cross-linker, intermolecularly and intramolecularly, forming covalent bonds for interconnecting the collagen fibrils, as well as polymerisation of glutaraldehyde to increase the network density [72].

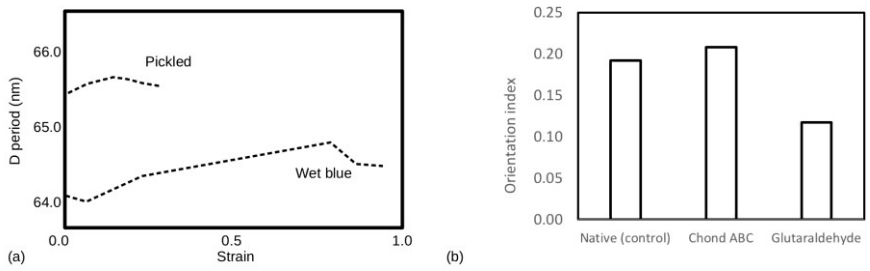

Fig. 6. Chemical treatment of collagen. (a) Sketch of graph of D period versus applied strain to partially processed skin [8] (Reprinted (adapted) with permission from Sizeland KH, Edmonds RL, Basil-Jones MM, Kirby N, Hawley A, Mudie S, Haverkamp RG: Changes to Collagen Structure during Leather Processing. Journal of Agricultural and Food Chemistry 2015, 63(9):2499-250. Copyright (2015) American Chemical Society) and (b) orientation index of native tissue versus tissue treated to Chondroitinase ABC (to remove GAGs) and glutaraldehyde (to introduce synthetic cross links), under tension. The values for the respective treatment were estimated from plots derived from a study reported elsewhere [72].

Alternatively, it has been proposed that collagen fibrils may interact directly without the help of extrafibrillar molecules by attributing the interaction to fibril branching [35]. During development, collagen fibrils grow in diameter and in length through both end-to-end and lateral fusion resulting in fibril branching; thus fibril branching is also regarded to facilitate interfibrillar load transfer between the small and large diameter fibrils [35]. Given that most tendons exhibit a distribution of small and large fibril diameters [55] small diameter fibrils may play an important function to connect and transmit force between the larger load-bearing fibrils in tendon [35].

3.4 Molecular level

At the molecular level, the mechanical stability of the fibrous structure may be better understood from studies of the assembly of the tropocollagen molecules into fibrils, which is regarded as a thermodynamically (entropy) driven process under ordinary/physiological conditions [73]. Tropocollagen molecule resembles a triple helical arrangement of three coiled collagen-protein chains, linked together by hydrogen bonds. When the molecule is stretched, at low displacement, the force generated in the molecule is low as the helix becomes uncoiled, but as the displacement increases, the force increases. These mechanical characteristics and structural bonding provide the cornerstone for understanding the ability of fibrous structures such as collagen fibrils to take up stress when stretched.

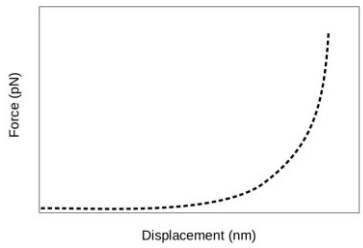

Fig. 7. Sketch of force versus displacement of tropocollagen molecule according to the worm-like-chain model.

The overall mechanical response of the molecule may be described by a worm-like chain (WLC) model [74] (**Fig. 7**). This force-displacement profile may be divided into three regimes, the low (near-constant) force regime is known as the entropic elastic regime while the linear regimes comprised of a low stiffness regime where the tropocollagen molecule continues to uncoil and a high stiffness regime where the molecule is fully stretched over the 'back-bone' so that further

stretching will rresult in rupture [75]. The mechanical stability of the molecule is parameterized by the molecular contour length and the persistence length [76] of the molecule at a predetermined absolute temperature. For a more detailed review of other modified WLC models (namely, extensible WLC, modified Marko-Siggia WLC, piecewise defined extended WLC, implicit elasticity WLC and twistable WLC) to described the highly stretched regime, see Hillgarten's report [77]. One important highlight of these variant models is that they are underpinned by different contour lengths and persistence lengths.

How the changes at the molecular level, namely the D period in collagen, contribute to bulk level behaviour such as dehydration, tanning, and stretching, have been reported in several studies. You may need no reminding that in native tissues tropocollagen molecules in fibrils are staggered axially, resulting in a periodic light-dark bands with a D period of about 67 nm when viewed under an electron microscope. The light bands are associated with gaps (region of low-density collagen packing) between the ends of two molecules, while the dark bands arise from molecular overlaps [78, 79]. The nature of the D period has been well-explored for a long time using data from x-ray diffraction peak patterns of hides [80]. For instance, Professor Eckhart Heidemann has probed the x-ray diffraction peak patterns of hides and found that the intensity varied with water content, which may be attributed to changes in the crystallinity of the collagen molecular packing, namely at the side-chain spacing [80]. Recently, new studies carried out to exploit the sensitivity of the intensity of these diffraction peaks for investigating the effects of the respective tanning agents BCS, ZIR, or ALS in *post-tanning*, have revealed how the metal ions from the respective tanning agents penetrate into the fibrils and interact with the collagen [81]. Other tanning agents such as fat liquor can penetrate into the fibril and interact with the molecules to change the D-period [80]. Overall, D period is shown to decrease with progressive leather processing stages [81]. With regard to dehydration studies, it is well-known that the D period decreases on drying [17, 82]. The reduced D period reflects the overall reduction in the characteristic gap (where collagen packing density is low) and overlap regions, possibly associated with deformation of the collagen crystal structure [17, 82]. Upon re-hydration, swelling of the fibrils occurs but a critical point is reached beyond which the fibril volume remains constant [43]. Thereafter, only the interfibrillar matrix continues to swells [43]. In some, but not all, species, the D period is also dependent on the location in the leather (through-thickness), such as the corium and grain layers [49]. However the extent of the differences with respect to the location may be species-dependent [83]. Mechanical deformation of leather materials can influence the D period, which is likened to an internal strain gauge [72]. The D period increases with increase in the tensile strain of leather [83] as the tropocollagen molecules elongate, and slip with respect to adjoining molecules, along the fibril axis, which changes the length of the gap-overlap regions [83, 84].

It is important to emphasize that large-scale changes in a collagenous material, such as leather stretching from a relaxed state or past the point of yielding, cannot be properly understood in terms what a single tropocollagen molecule is doing using the WLC model, although the WLC is a useful model for understanding how a molecule responds to an external load. Current interest in multiscale modelling of the collective 'many-molecule' behaviour (e.g. by incorporating the WLC model) at the molecular level, the collective 'many-fibril' behaviour (e.g. by incorporating stress transfer mechanisms) at the fibrillar level, and the correlations that must be established between the different levels across the full length scale as reported in several fundamental papers [75, 85-92], may be the answer to understanding the stability of collagen in leather. To address this approach would require establishing a conceptual framework underpinning organized information of the structure-function relationship of collagen. This is highlighted in the following (final) section of this paper.

4 Hierarchical architecture of collagen

Section 0 to 0 highlight the key findings on collagen stability from interfibrillar matrix level to tropocollagen molecular level. But can we fit these seemingly disparate pieces together to help us see the bigger picture better? In 2014, I proposed a strategy to help advance our understanding of the structure-function relationship of ECM. This strategy underpins a concept of organized information addressing a framework for the mechanisms of stress uptake in the structural units reinforcing the tissue at the respective levels of the hierarchical architecture [59]. The framework that I have constructed takes the form of a table initially but this eventually led to a schematic of structural levels versus loading stages, regulated by known mechanisms at each structural level and corresponding loading stage. A schematic of the framework is shown in **Fig. 8**. The framework was aimed at facilitating (1) comparison of individual stress uptake mechanisms between different tissue structural components of the same structural levels and across different structural levels, (2) comparison of mechanical pathways, and (3) prediction of new interconnection between existing mechanisms. A detailed description of the individual stress uptake mechanisms, mechanical pathways and interconnections is reported elsewhere [59, 93].

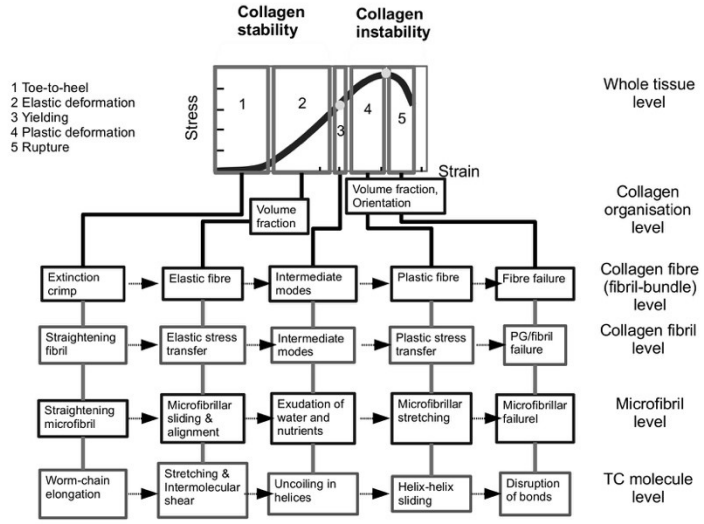

Fig. 8. Stress transfer mechanisms in ECM at various structural levels from macro to nano scale. The schematic diagram, proposed by Goh et al. [59], addresses both the structural organisation at different lengthscales and the different loading stages from initial until fracture. One may view the schematic diagram from left to right and from top to bottom. For the purpose of referencing, the structural lengthscales are labelled 1-6 and the stages of loading are labelled 1-5. The Inset shows a sketch of graph of load versus strain for (e.g. in vitro, uniaxial tensile test) of dermal skin, accompanied by schematics of the skin showing the collagen fibre orientation at various stages of the loading process. In a, collagen fibres appear disorganised with regards to direction, but becoming more aligned along the direction of the load acting on the skin. As the load increases further, the proportion of the collagen fibres becoming more aligned (and also straight) increases (part c). Finally, all collagen fibres are almost aligned and straight [94].

The framework for describing the hierarchical architecture can be applied to leather by organizing findings from experiments and predictions from analytical/computational models in leather studies. The findings would serve to aid researchers' understanding of the structure-function phenomena or to inform manufacturing decisions with socioeconomic consequences.

While many studies on collagen fundamentals have used computational biomechanics models such as those proposed by Buehler and co-workers [75, 86-88, 95-98], there is a dearth of reports from analytical/computational modelling in leather studies. It is likely that increased computer speed and better specialist software will enable collagen modelling, i.e. *in silico* leather, to be carried out, to be applied to an increasingly wide range of problems, and to be deployed in the manufacturing of leather where modelling could be incorporated as part of the process. On this

note, it is important to deal with model credibility where computational biomechanics models would be used for leather properties predictions. One such approach proposed by Patterson and Wheelan[99]-which is a simple 3x3 matrix for facilitating the categorization of models with respect to their testability-may be employed in order to guide the selection of an appropriate process of validation so that the leather researcher can obtain the evidence to establish credibility.

5 Conclusion

This paper has discussed recent findings on the role of collagen in regulating the mechanical stability of biological materials, covering three areas: physicochemical factors affecting collagen stability, mechanics of collagen stability and cross-links, and the hierarchical architecture of collagen. The recent findings highlighted here are likely to encourage further development of leather that addresses collagen stabilization and achieve a more sustainable future for the leather industry.

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