



Probing multi-scale mechanics of peripheral nerve collagen and myelin by X-ray diffraction

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ABSTRACT

Peripheral nerves are continuously subjected to mechanical forces, both during everyday movement and as a result of traumatic events. Current mechanical models focus on explaining the macroscopic behaviour of the tissue, but do not investigate how tissue strain translates to deformations at the microstructural level. Predicting the effect of macro-scale loading can help explain changes in nerve function and suggest new strategies for prevention and therapy.

The aim of this study was to determine the relationship between macroscopic tensile loading and micro scale deformation in structures thought to be mechanically active in peripheral nerves: the myelin sheath enveloping axons, and axially aligned epineurial collagen fibrils. The microstructure was probed using X-ray diffraction during *in situ* tensile loading, measuring the micro-scale deformation in collagen and myelin, combined with high definition macroscopic video extensimetry.

At a tissue level, tensile loading elongates nerves axially, whilst simultaneously compressing circumferentially. The non-linear behaviour observed in both directions is evidence, circumferentially, that the nerve core components have the ability to rearrange before bearing load and axially, of a recruitment process in epineurial collagen. At the molecular level, axially aligned epineurial collagen fibrils are strained, whilst the myelin sheath enveloping axons is compressed circumferentially. During induced compression, the myelin sheath shows high circumferential stiffness, indicating a possible role in mechanical protection of axons. The myelin sheath is deformed from low loads, despite the non-linearity of whole tissue compression, indicating more than one mechanism contributing to myelin compression. Epineurial collagen shows similar load-bearing characteristics to those of other collagenous connective tissues.

This new microstructural knowledge is key to understand peripheral nerve mechanical behaviour, and will support new regenerative strategies for traumatic and repetitive injury.

1. Introduction

Peripheral nerves are continuously subjected to mechanical forces, elongation, and compression during everyday movement, without suffering functional losses and damage. Traumatic events and inappropriate continuous mechanical loading, however, are associated with common disabling and painful entrapment, overstretch, or compression neuropathies. Carpal tunnel syndrome, for example, has a prevalence in the United Kingdom of 5–16% annually, varying by age and gender group, with decompression surgery provided for 43–74 people per 100,000, annually (Aroori and Spence, 2008; Burke, 2000). Erb's palsy, caused by excessive stretching of infant heads and arms during birth, induces loss of sensation and abnormal motor function in

0.1% of births in the US (Gilbert et al., 1999). In sciatic nerves and brachial plexus, stretch due to trauma and abnormal limb positioning during operations are widespread causes of debilitating iatrogenic injury, both temporary and chronic (Lalkhen and Bhatia, 2012). Mechanical tension has also been proposed as a regenerative method, with mild stretch inducing axonal and whole-nerve elongation and growth, although the multi-scale effects of this elongation have not been studied (Pfister et al., 2004; Chuang et al., 2013; Saijilafu et al., 2008). A better understanding of the multi-scale link between macroscopic loading and loss of function in peripheral nerve is required for effective prevention and treatment of neuropathies, and to explore tension as a strategy for injury prevention and regeneration (Bueno and Shah, 2008).

Multiple concurrent factors cause functional alterations in

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peripheral nerves. Occlusion of nerve blood supply, leading to hypoxia, occurs at macroscopic strains above 15% (Ogata and Naito, 1986). A reduction in electrical conduction has also been observed as a result of stretch, with Compound Action Potential magnitude decreasing with increasing strain (Li and Shi, 2007; Wall et al., 1992), and completely subsiding at strains of 5–20% (Li and Shi, 2007; Takai et al., 2002). At a cellular level, strains above 10% have been shown to affect axonal transport, responsible for motility of cytoskeletal elements, energy production, and growth factor trafficking (Ikeda et al., 2000; Aomura et al., 2016), and AFM studies on single axons showed that the compression required to block axonal transport was variable, between 65 and 540 Pa (Magdesian et al., 2012). Furthermore, voltage-gated sodium channels clustered at Nodes of Ranvier, fundamental for saltatory impulse conduction, have been shown to disperse with applied nerve elongation (Ichimura et al., 2005). Axonal tensile strains of 5% can cause growth cone collapse (Yap et al., 2017), membrane permeabilisation (Geddes et al., 2003), and morphological changes in neurons (Kilinc et al., 2008), also potentially leading to loss of function.

The mechanical properties of peripheral nerve tissue derive from its complex structural organisation. Substructures in peripheral nerves include the endoneurium, a loose structure of collagenous channels in which myelinated and unmyelinated axons are embedded, surrounded by the perineurium, made up of multiple layers of transversely aligned lamellar collagen. The epineurium, a thick layer of densely packed collagen fibres, envelopes the whole nerve in an axially aligned pattern, showing fibril waviness and crimp (Fig. 1) (Ushiki and Ide, 1990). The properties of these collagenous substructures have been shown to be similar to those of tendon (Mason and Phillips, 2011) and either or both the epineurium and perineurium have been described as the main load bearing structures (Sunderland and Bradley, 1961; Haftek, 1970; Rydevik et al., 1990; Tillett et al., 2004). Here, we analyse the partitioning of strain between whole tissue and collagen molecules, to compare the mechanical properties of axially aligned epineurial collagen to those of other load-bearing collagenous tissues such as tendon.

Mechanically, peripheral nerves have been modelled as concentric, two-layer composites: a more compliant, water-rich core representing endoneurium, the inner part of the perineurium, and myelinated and unmyelinated axons, and a stiffer outer sheath representing the epineurium and the outer perineurium, connected by a sliding interface layer (Tillett et al., 2004; Georgeu et al., 2005; Walbeehm et al., 2004). Independent mechanical testing characterises the core as significantly softer and more compliant than the sheath, and indicates that, during tensile loading of the nerve, the outer sheath applies a compressive force on the core, which is resisted by a positive endoneurial pressure (Walbeehm et al., 2004; Georgeu et al., 2005). The loose organisation of endoneurial tissue suggests that, at low loads, components within the

core rearrange, rather than compress, during whole tissue compression (Millesi et al., 1995). The transverse compression induced by tensile loading has been observed *in vitro*, but its effect on the microstructural elements within the core has not been studied (Topp and Boyd, 2006; Millesi et al., 1995).

Within the nerve core, chemical demyelination has been shown to reduce nerve axial stiffness, implying that myelin contributes to tissue mechanical properties (Shreiber et al., 2009). Additionally, AFM studies have shown that digestion of Schwann cell basal lamina reduces nerve fibre circumferential stiffness and resilience significantly (Rosso et al., 2014), suggesting a protective role for myelin during nerve deformation. However, the mechanical role of myelin is not yet clear, as it has mostly been studied in isolation, rather than within the multi-scale mechanical environment of the whole nerve. Here, we aim to characterise the mechanical properties of the myelin sheath during circumferential compression induced by whole-nerve elongation.

Linking macroscopic loading of peripheral nerves with changes in cell function and damage requires knowledge of the multi-scale mechanical behaviour of nerves. Current knowledge of the micro-scale effects of macroscopic loading is limited, and a better multi-scale understanding is required to successfully prevent and treat mechanically-induced peripheral neuropathy and functionality loss (Bueno and Shah, 2008). X-ray diffraction is an ideal modality for *in situ* strain measurements of micro-scale quasi crystalline structures. In nerve, it has been shown to effectively probe collagen fibres (Inouye and Worthington, 1983) as well as the intra-lamellar spacing in the myelin sheath (Finean, 1960; Inouye et al., 2014; Kurg et al., 1982), but it has not been applied to studying the mechanical properties of these structures during *in situ* loading. Here we investigate the multi-scale mechanical properties of peripheral nerve during tensile loading, by probing the microstructure of peripheral nerve collagen and myelin by X-ray diffraction during macroscopic tensile loading.

2. Materials and methods

2.1. Sciatic nerve harvesting and imaging

Sciatic nerves were harvested from 300 to 350 g (10–12 week old), male Sprague-Dawley rats, sacrificed by cervical dislocation for an unrelated study. Briefly, following skin removal, the sciatic nerves were exposed by dorsal incision of the gluteus muscle. Nerves were excised proximally close to the spinal cord, and distally at the tibial-peroneal branching. Nerve samples were stored in Phosphate Buffered Saline (PBS, Gibco, UK), immediately frozen at -20° until use. This has been previously shown not to alter the mechanical properties of collagenous tissues (Bruneau et al., 2010; Fessel et al., 2014), as well as

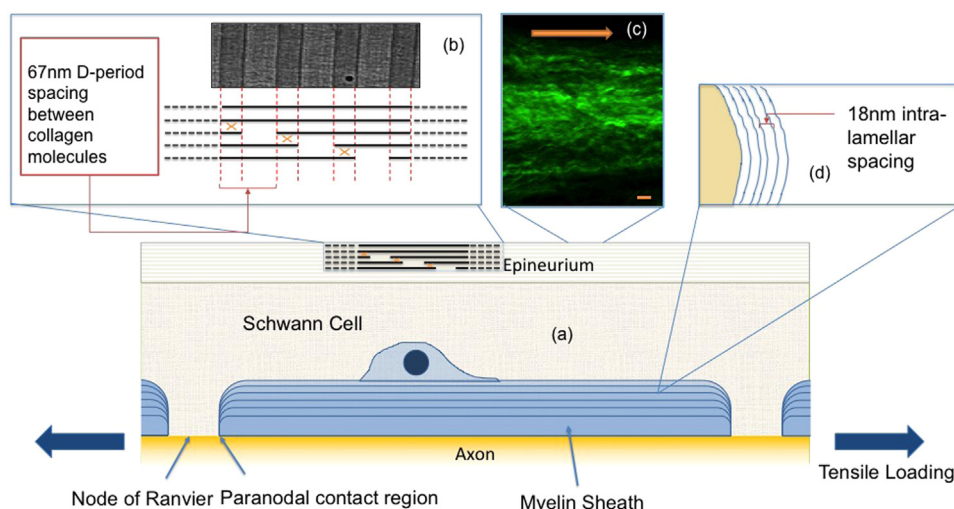


Fig. 1. (a) Peripheral nerves consist of axons projecting from neuron bodies, which can be myelinated by Schwann cells or unmyelinated. The structure of quasi-crystalline elements (collagen and myelin) can be observed by X-ray diffraction. (b) Epineurial, axially aligned collagen fibril structure, with characteristic 67 nm D-period between molecules. (c) Multi-photon second harmonic microscopy of rat sciatic nerve collagen, showing crimped wavy pattern of axially aligned fibres. Scale bar = 100 μ m. Arrow indicates direction of nerve axis. (d) Cross-sectional view of myelin sheath, showing 18 nm spacing between lamellae.

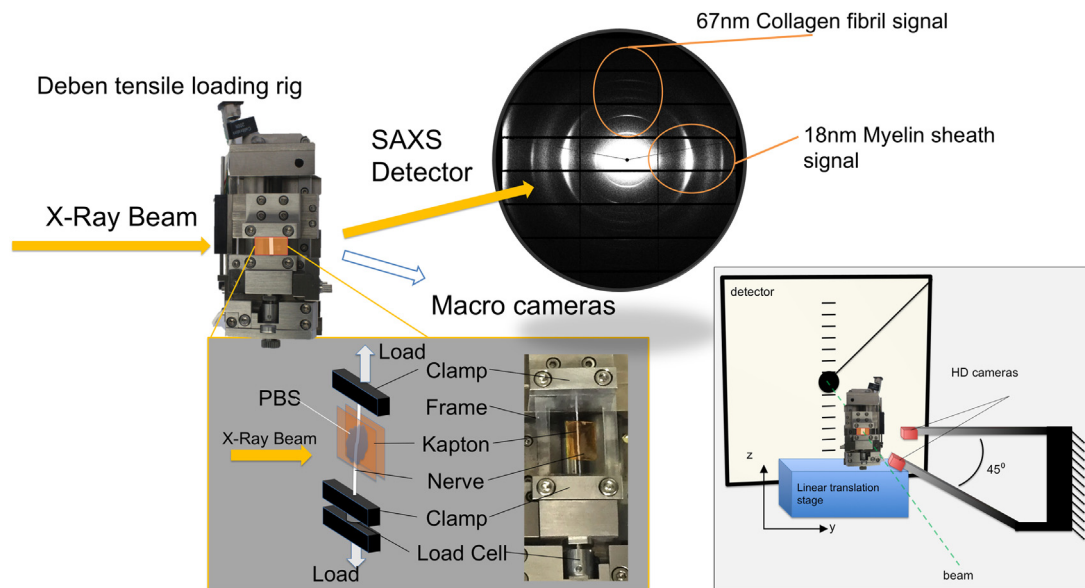


Fig. 2. Experimental setup and data acquisition. X-ray beam is diffracted by nerve sample loaded in tension by Deben rig. Kapton film and PBS keep the samples under physiological hydration conditions. Small angle scattering of X-rays is detected, showing peaks corresponding to intra-molecular spacing in epineurial collagen fibrils, and peaks corresponding to the intra-lamellar spacing in the myelin sheath. Inset shows diagrammatic view of camera setup, as seen from the X-ray source.

retraction properties of sciatic nerves (Walbeehm et al., 2004). Furthermore, Wallarian degeneration of axons and the myelin sheath has been shown to be delayed by low temperatures (Sea et al., 1995). Together, these suggest that freezing nerves upon excision and thawing prior to experiments should retain the tissue mechanical properties. After thawing and mounting, ink marks were made on samples, for optical measurement of whole tissue strain as previously used for spinal cord and tendon strain measurements (Shreiber et al., 2009; Bianchi et al., 2016). To highlight the crimped epineurial collagen structure, nerves were imaged using multi-photon second harmonic generation (SHG) imaging using a Zeiss LSM780 microscope (20x lens), with excitation set at 880 nm.

2.2. In situ loading

Sciatic nerves were loaded *in situ* to failure (defined as complete tissue disconnection) using a Deben Microtest (Deben, UK) tensile loading stage, equipped with a 2 N load cell. Samples were glued to a rectangular plastic frame using cyanoacrylate glue (Loctite, UK). Frames were mounted in the Deben stage by clamps (Fig. 2). Before experiments, the sides of the frames were cut, leaving the nerve as the only load bearing element between the clamps. Two rectangular sheets of Kapton film (Goodfellows, UK) film were placed either side of the nerves, and PBS was added to the samples to maintain physiological hydration, and to hold the Kapton film together by capillary action. The loading rig was mounted vertically, and rotated by 45° to avoid blocking detectors. Two cameras were placed, facing the sample, at 45° relative to each other (Fig. 2, inset).

For 6 sciatic nerve samples, increasing tensile loads were applied, and diffraction patterns recorded at approximately 0.1 N intervals. In order to avoid creep-like effects, X-ray diffraction images were taken during continuous loading, at the instant when the load cell indicated the required load. Loading was assumed to be constant during acquisition, as a quasi-static loading at 0.1 s⁻¹ strain rate was used, much slower than acquisition time.

2.3. X-ray diffractometry

Diffraction experiments were carried out at beamline I22, Diamond Light Source, UK. The X-ray beam dimensions were approximately

300 × 100 μm² FWHM Horizontal × Vertical, with the largest dimension parallel to the sample axis (z-direction in Fig. 2, inset). A photon energy of 12.4012 ± 0.0014 keV was selected ($\frac{dE}{E} = 1.12 \times 10^{-4}$), corresponding to an X-ray wavelength of 0.0997 ± 1.0066 × 10⁻⁵ nm. SAXS data was collected over a q range of 0.0026 – 0.13 Å⁻¹, and over an azimuthal range of 360° using a 2D Pilatus P3-2M detector (DECTRIS, Switzerland) placed 6.539 m downstream of the sample and with a typical exposure time of 0.15 s. Beam centre on the detector was determined using the Debye-Scherrer rings from Silver Behenate, and the sample-detector distance was determined using the diffraction from chicken tendon collagen standard reference samples.

Pilot experiments showed small angle X-ray scattering (SAXS) of peripheral nerve with a highly textured, axially aligned collagen diffraction pattern, with peaks corresponding to multiple diffraction orders of the 67 nm axial spacing (D-spacing) between molecules within fibrils (Fig. 1b), whilst myelin diffraction produced equatorial peaks at 90° orientation to the collagen, measuring the 18 nm average spacing between lamellae (Fig. 1c).

2.4. X-ray diffraction pattern analysis

SAXS diffraction patterns were converted from Cartesian detector coordinates to polar coordinates, showing diffraction data as a function of azimuthal angle, χ , and scattering vector magnitude, q (Fig. 3). The geometrical parameters of the experimental setup, essential for this remapping, were determined by calibration SAXS measurement of chicken tendon collagen standard reference samples. Gaussian profiles were fitted to the 1st, 3rd, 5th and 9th order meridional collagen peaks, and their maxima used to determine peak position. Similarly, myelin diffraction patterns were extracted by fitting Gaussians to two equatorial peaks from the same images.

Q-scale peak positions were then divided by the diffraction peak order number, converted to D-spacing measurements, and averaged over the number of fitted peaks. Nominal strain was measured by the displacement of average peak position relative to an unloaded state, using Eq. (1), where X can refer to any spacing parameter observed, and X₀ being the value of the parameter in the unloaded state.

$$\epsilon_X = \frac{X - X_0}{X_0} \quad (1)$$

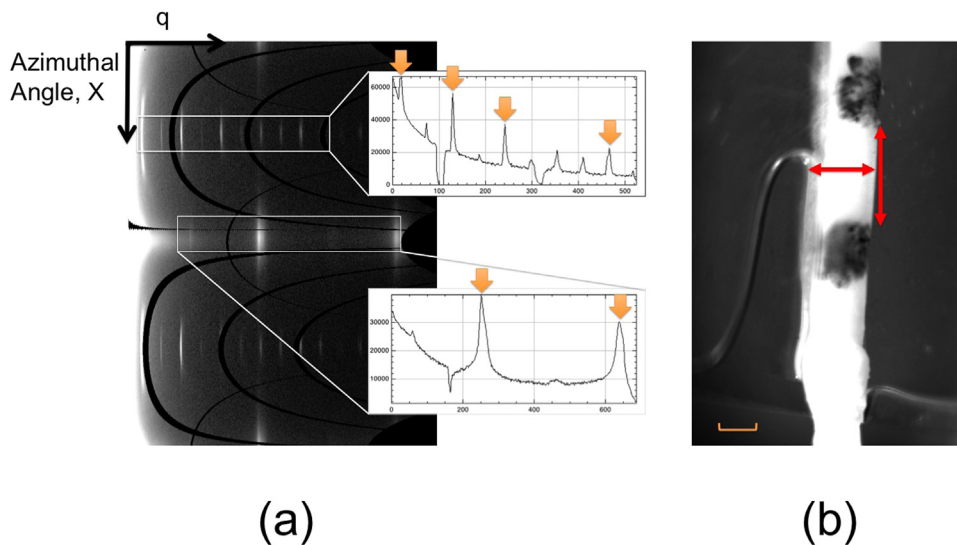


Fig. 3. (a) Small angle diffraction pattern from rat sciatic nerve, following conversion from Polar to Cartesian coordinates, showing characteristic peak patterns from collagen (top) and myelin (bottom). Yellow arrows show fitted peaks after line profile plotting. (b) Camera image for video extensimetry measurement, showing black marks used for tissue strain computation. Arrows show transverse and axial lengths measured. Scale bar = 1 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Stress-strain data was calculated only for axial tissue and collagen deformation. Stress values for collagen were calculated assuming that the epineurium constituted 50% of nerve cross-sectional area (Flores et al., 2000). For all other measurements where area could not be approximated, data was presented as load against strain plots, showing material stiffness. Load, being the controlled variable, is plotted on the horizontal axis, unlike in stress-strain curves, as previously used to characterise the mechanical properties of other collagenous tissues (Oxlund et al., 2010). Linear and non-linear regression and curve fitting is carried out using GraphPad PRISM. Non-linear fitting is used for macroscopic tissue strains, where non-linear relationships between applied force and elongation have been previously reported (Millesi et al., 1995; Walbeehm et al., 2004).

2.5. Whole tissue strain calculations

Strain in the whole tissue was measured axially and transversely from images taken by a HD camera (AlliedVision, Germany). Axial tissue strain was estimated using marker tracking of the black surface ink marks. A custom MATLAB code was used, which estimates strain by extracting centroid coordinates of the ink marks and calculating the change in distance between marks. This was validated by manual measurement of the distance between marks. In the transverse direction, contraction with applied load was measured by extracting nerve diameter from images, by measuring full width at half maximum of line profiles across the nerve. The nerve was split into 50 axial segments, and the diameter calculated as the average of the diameter value measured in each section. Tissue strain measurements and relation to molecular strains assume circular geometry and symmetry.

3. Results

3.1. Tissue and molecular strains

With increased tensile load, nerve tissue elongates axially, with simultaneous circumferential compression (Fig. 4a and b). In the axial direction, the tissue extends at a faster rate at lower loads, a non-linear behaviour (average $R^2 > 0.87$) consistent with previously published results (Millesi et al., 1995; Topp and Boyd, 2006). Circumferential compression is also non-linear, with a higher rate of compression at lower loads, and with strain reaching a limiting value at higher loads (average $R^2 > 0.9$), consistent with the hypothesis that internal core components rearrange before being loaded during induced compression (Topp and Boyd, 2006; Millesi et al., 1995).

Loading modulus (calculated from the linear section of the loading curves) for whole nerves was 5.31 ± 2.55 MPa, in accordance with previously reported values for sciatic nerve stiffness (Topp and Boyd, 2006). Assuming epineurial thickness to be 50% of nerve volume (Flores et al., 2000), loading modulus of epineurial collagen fibrils was 92.34 ± 35.01 MPa, lower than that of rat tail tendon collagen fibrils measured by X-ray diffraction (Bianchi et al., 2016), but in line with other reported moduli for collagen fibres (Sasaki and Odajima, 1996).

With increasing applied load, the average molecular D-spacing measured in collagen increased linearly in all samples (average $R^2 > 0.9$) up to strains of 2% (Fig. 5a). Concurrently, average spacing between lamellae in the myelin sheath decreased, reaching strains of up to -0.6% (Fig. 5b).

3.2. Strain partitioning between length scales

Partitioning of strain can be measured in both collagen and myelin (Fig. 6). Tissue level axial strain exceeds collagen fibril strain, indicating the existence of multiple mechanisms for tissue extension in addition to collagen fibril elongation, as reported for other collagenous connective tissues (Fratzl et al., 1998; Bianchi et al., 2016). Tissue circumferential compressive strain is also larger than the myelin layer compression suggesting that the myelin sheath is significantly stiffer than its surroundings. Average myelin stiffness is higher than circumferential tissue stiffness, with a ratio of the slopes of linear portions of load-strain curves of 100 (Fig. 6b). For collagen, the same slope ratio is 10 (Fig. 6a), indicating that collagen bears a higher proportion of axial load than the proportion of circumferential compression borne by myelin.

The difference between tissue and molecular strain at each loading point represents straining due to mechanisms other than molecular deformation, such as molecular sliding and structural rearrangements. Axially, rearrangements in collagen structure account for more than 90% strain at lower loads, and molecular elongation increases at higher loads. Circumferentially, myelin compression accounts for less than 2%, on average, of tissue compression.

Macroscopically, axial tissue strain correlates linearly (average $R^2 = 0.87$) with collagen strain (Fig. 7a), indicating a direct mechanical relation between the two length scales. Variations in slope of tissue strain to collagen strain relations are due to the higher variability of tissue strain measurement, as previously shown for whole tendon tissue strains measured using a similar technique (Bianchi et al., 2016). Circumferentially, no linear correlation is observed between molecular and tissue strain (Fig. 7b), suggesting that induced macroscopic

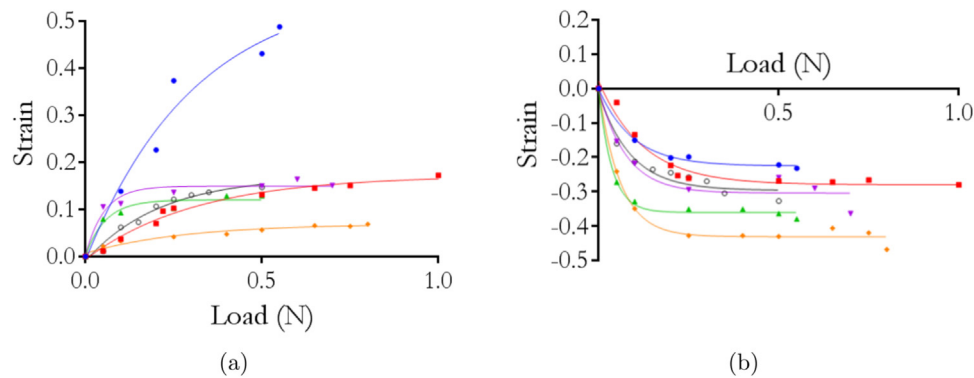


Fig. 4. Tissue-level strains as a function of load in rat sciatic nerves (N = 6). Axial tissue strain (a) is positive and increasing with load, circumferential strain (b) is negative (compression) and increasing in magnitude with load. Both deformations are nonlinear with applied load.

compression is not the only mechanism contributing to myelin deformation.

4. Discussion

Using X-ray diffraction and HD video extensimetry during *in situ* tensile loading, we have investigated the multi-scale mechanical properties of peripheral nerves. By using X-ray diffraction, molecular-level mechanical behaviour was observed in both axially-aligned epineurial collagen, and in the myelin sheath enveloping axons. Results presented show a pronounced circumferential compression in rat sciatic nerves during axial elongation. At a molecular level, the spacing between myelin lamellae decreases, with indication of high compressive stiffness of myelin, and epineurial collagen elongates, showing similar properties to those of load-bearing connective tissues.

A pronounced macroscopic circumferential compression is observed during tensile loading of sciatic nerves. This supports previously proposed models, where a compression of the nerve trunk occurs during tensile loading (George et al., 2005; Topp and Boyd, 2006; Millesi et al., 1995). Evidence of epineurial thickness remaining constant during elongation (Islam et al., 2012) indicates that compressive strain is localised in the core. The loose organisation of perineurial and endoneurial tissue suggests that components within the core can rearrange, rather than compress, with decreasing nerve diameter (Millesi et al., 1995). The non-linear behaviour observed here at tissue level (Fig. 4b) confirms the presence of softer structures that allow rearrangement under small loads, and stiffer structural elements contributing to the behaviour at higher loads.

Our results show that, when a peripheral nerve is elongated axially, the average spacing between lamellae in the myelin sheath decreases linearly (Fig. 5b). This suggests that myelin is being deformed

throughout the loading curve, both at lower loads, during rearrangement of softer structures, and at higher loads, where the nerve exhibits higher circumferential stiffness. No direct correlation between axial tissue compression and myelin compression is observed (Fig. 7b), indicating that the circumferential compression is not the only mechanism causing myelin compression.

Myelin has been shown to have significant compressive stiffness (Rosso et al., 2014; Shreiber et al., 2009). The observed behaviour can be explained if myelin compression is not solely caused by tissue compression. Previously, it has been shown that axial elongation of myelinated nerve fibres is both due to a lengthening of the internodal region (Maxwell, 1996; Yokota et al., 2003), as well as a more prominent widening of the Nodes of Ranvier (Kerns et al., 2001; Ikeda et al., 2000; Ichimura et al., 2005). This suggests that axons are being loaded in tension, and that the myelin sheath, despite contributing to tensile stiffness, is being elongated with the axons. The myelin sheath is tethered to the axons it surrounds through contact proteins located at the paranodal region. If axons are also loaded in tension, the spacing between myelin lamellae would decrease, as it cannot slide along the axons (Fig. 8), explaining behaviour at low loads (Inouye et al., 2014). At higher loads, the stiff myelin sheath is further compressed with induced circumferential strain, but acts as a stiff protective layer to reduce potentially damaging axonal compression.

When peripheral nerves are loaded in tension, axially aligned collagen fibrils present in the epineurial outer sheath also extend, showing a linear relation between axial tissue strain and collagen molecular strain (Fig. 7a). In tendon collagen, a load-bearing energy storing structure of similar fibrillar morphology and density (Ushiki and Ide, 1990) subjected to continuous physiological tensile loading, accepted deformation mechanisms accounting for differences in molecular and tissue axial strain include both geometric rearrangement of collagen

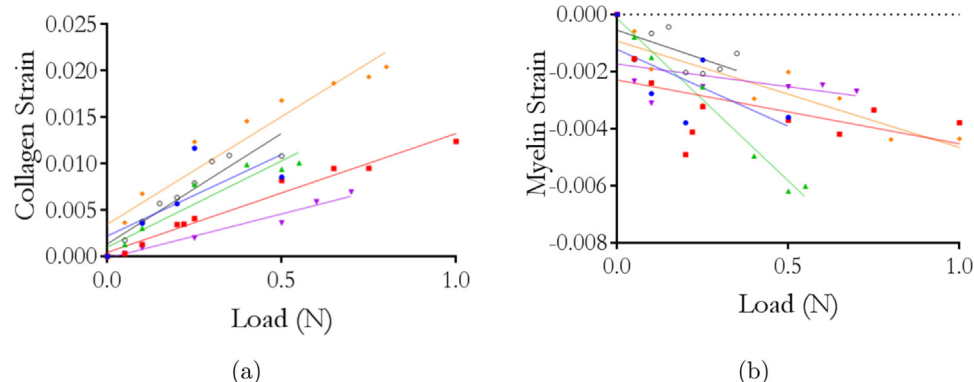


Fig. 5. Molecular-level strains in collagen axial (a) and myelin circumferential (b) as a function of load in rat sciatic nerves (N = 6). In the collagen matrix, a positive strain (elongation) is observed. In the myelin sheath, a negative strain (compression) is observed.

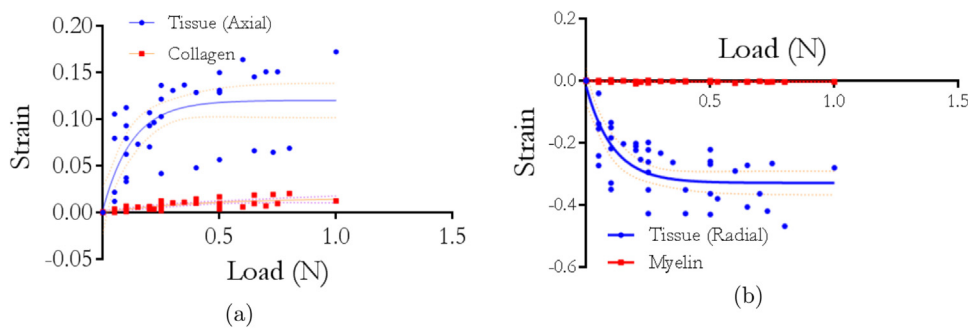


Fig. 6. Strain partitioning between molecular (red) and tissue (blue) levels, for collagen (a) and myelin (b). In both cases, straining of the observed molecular structure only accounts for a fraction of the total tissue strain in the same direction. Lines correspond to non-linear fit as shown in Fig. 4. Dotted lines = 95% confidence interval. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

fibrils (uncrimping) and relative sliding of discontinuous fibrils (Bianchi et al., 2016; Fratzl et al., 1998). Similarly, when a peripheral nerve is stretched, part of this elongation is due to extension of the axially aligned epineurial collagen fibres, and part is due to molecular rearrangements and deformation of other components. We have shown that strain due to factors other than collagen molecular straining are more dominant at lower loads, and vary non-linearly with applied load. This is in accordance to models of collagenous materials which include fibril recruitment, where wavy fibrils are first recruited, and start bearing a higher proportion of load once they have been fully straightened (Thompson, 2013). This also agrees with mechanical testing performed on separated nerve cores and sheaths, where the outer layer has been shown to bear load only after an initial toe region (Tillet et al., 2004). Loading modulus of epineurial collagen measured here is smaller than that reported from X-ray diffraction studies on rat tail tendon collagen (Bianchi et al., 2016), suggesting that other elements of the peripheral nerve, such as perineurial collagen and axons, bear a fraction of the applied tensile load, but confirming that the epineurium has a vital mechanical role during tensile loading. The large inter-species variability, as well as the differences in mechanical properties between functionally specialised tendons may also contribute to the difference measured (LaCroix et al., 2013).

4.1. Limitations of this study

The study presented here is limited by the small sample number, and by the elements that are observable using X-ray diffraction. Direct information about the deformation of axons, blood vessels and other structures within the nerve would provide further explanation for the mechanisms of deformation, and could lead to a better quantification of induced compressive strain partitioning. Previous work on tracking axonal strain using surface protein markers suggests a technique which could be employed (Singh et al., 2017). This study is further limited by the lack of information about volume fraction of materials present in peripheral nerves. This information would allow stiffness of nerve

collagen and myelin to be better estimated. Another limitation is the absence of information about myelin properties in tension, which would further refine the whole-nerve model. The loading methods should be considered as a factor further limiting these results. *In situ* tensile loading does not precisely reproduce *in vivo* nerve loading conditions, where the nerve is not clamped at both ends. This could affect the way in which nerve core elements are strained. Furthermore, a thorough characterisation of the differences between freshly excised tissues and frozen tissues would provide stronger evidence that sample preservation did not affect its mechanical properties.

This study could be the base for further studies to observe how larger deformations translate to the micro-scale, and damage peripheral nerves, as well as to study the effect of demyelinating disease models on the mechanical behaviours described here.

5. Conclusions

In this study, we probe the micro-mechanical behaviour of peripheral nerve collagen and myelin during *in situ* tensile loading, by X-ray diffraction. Results show a non-linear compression of the nerve induced by tensile loading, confirming previous hypotheses that soft nerve core elements rearrange before being loaded. We show that at the microscopic level, the spacing between lamellae of the myelin sheath decreases with applied whole-nerve tensile load, but myelin exhibits a much higher stiffness than the whole nerve. This suggests that myelin is mechanically significant in compression as well as in tension, protecting underlying axons from compressive damage.

Axially, we confirm strain partitioning in epineurial nerve collagen, similar to that observed in tendons, suggesting similar load-bearing properties.

These results have implications in understanding mechanical behaviour of peripheral nerve tissues, as well as mechanisms of cellular damage caused by macroscopic loading. Furthermore, understanding the microscopic nerve mechanical environment during mechanical loading is fundamental to devise appropriate strategies for injury

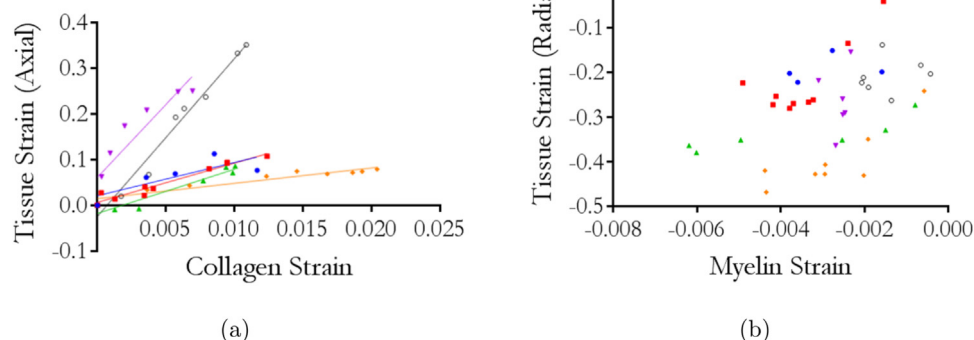


Fig. 7. Molecular strain increases linearly with tissue strain in axially aligned collagen (a), but shows no relationship axially during myelin compression (b) in rat sciatic nerves (N = 6).

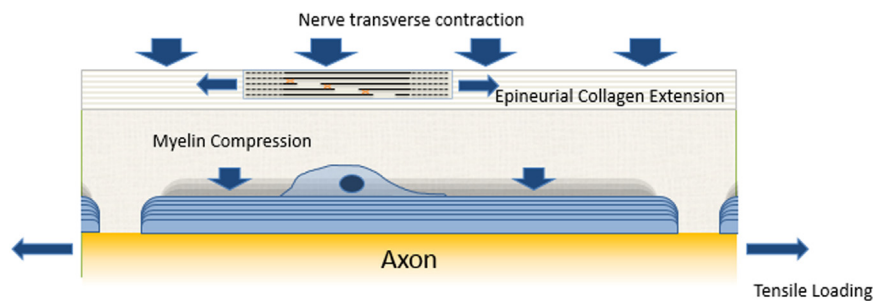


Fig. 8. Proposed mechanism of myelin compression as a result of nerve tensile loading. Epineurial collagen elongates axially, taking most of the load, with some load being taken by the axons and myelin sheath. The myelin sheath, which is tethered at paranodes, elongates with the axons, and is compressed circumferentially. The combined effect of induced tissue compression and axonal elongation induces a compression of myelin layers.

prevention and regeneration strategies (Bueno and Shah, 2008).

Further investigation, including analysis of axon deformation during whole nerve loading, is required to fully understand how the functional properties of nerve tissues are altered during mechanical loading.

Conflicts of interest

The authors declare that they have no conflict of interest.

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