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The biomechanical characteristics of spinal dura mater in the context of its basic morphology

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Purpose: Spinal dura mater plays a crucial role in the biomechanics and protection of the spine. Therefore, the present study investigated the dura mater's mechanical and basic morphological properties to learn more about the biomechanical behaviour of this fibrous membrane. *Methods*: Tissue strips, oriented in the longitudinal and circumferential directions, were cut from the cervical, thoracic, and lumbar vertebrae parts of the porcine spinal cord. Uniaxial tensile tests were performed using a device with a speed of 4 mm/min until rupture of the sample. *Results*: It was demonstrated that the dura mater is a heterogeneous, anisotropic material. The longitudinal excised specimens showed the highest values of mechanical properties (ultimate force (F_U), the stiffness coefficient (k), ultimate tensile strength (σ_{UTS}), and Young's modulus (E)) compared to those of the circumferentially. Confocal microscopy and sulforhodamine B (SRB) assay enabled us to visualise collagen and elastin elements more efficiently without a need for sample fixation. *Conclusions*: The spinal dura mater mechanical properties are not uniform along the entire length of the spinal cord, but, in the case of morphological features, no major differences were noticed. The utilisation of SRB occurred to be a non-destructive, fast, and efficient tool for visualising even the smallest elastic fibres on different depths of examined samples. The mechanical and morphological properties of the dura mater provided by this study can be further used in computational modelling to understand injury mechanisms better and help develop injury prevention strategies.

Key words: *spinal dura mater, mechanical properties, collagen and elastin fibres, confocal microscopy, sulforhodamine B, porcine model*

1. Introduction

The spinal cord, like the brain, cannot regenerate itself the way most other human tissues do. For this reason, spinal cord damage is often irreversible and may have severe repercussions. Damages of the spinal cord structure can be caused by various factors (e.g., neoplasia, inflammation), but mainly – as a result of mechanical injuries [19], [36]. The key role in protection, stabilisation, strength and, in general, biomechanics of the spinal cord is played by dura mater [9], [18].

The spinal dura mater is the continuation of cranial dura mater starting beyond the foramen magnum and brainstem. Still, it lacks an external endosteal layer in contrast to the cranial dura mater (it consists only of inner – meningeal layer). It is a strong tubular structure composed mainly of fibrous proteins (collagen and elastin fibres) and fibroblasts embedded in an amorphous extracellular ground substance [4]. The knowl-

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edge concerning the structure of the spinal dura mater could be very useful in designing medical devices that will directly interact with this membrane and inventing the best implantation material (fibrin sealants or dural patches, dural substitutes) [18]. Moreover, because dural punctures are a routine procedure for diagnostic and therapeutic purposes, it is essential to learn more about the response of the dural tissue to damages. Like other soft tissues, dura mater owes its tensile strength and elastic properties to its main fibrous proteins and, consequently, it can endure often high mechanical loadings. When tested to failure in uniaxial tension, the spinal dura mater demonstrates nonlinear stress–strain behaviour typical of collagenous soft tissues. Additionally, anisotropy of spinal dura mater has been identified both in animals [4], [16], [25] and humans [25]. The reason is preferentially longitudinal (cranio-caudal) arrangement of the collagen fibres [21]. Broadening the knowledge of the mechanical properties of the spinal dura mater, particularly in the context of its structure, helps to better understand its behaviour and interactions with the spinal cord after traumatic events. It may improve patient treatment and care after spinal cord injuries. It should be underlined, however, that accurate characterisation of strength parameters of spinal dura mater *in vitro* can be challenging, because of several variables such as storage conditions and time *postmortem* affecting tissue mechanical behaviour.

This study aimed to determine basic mechanical properties together with describing morphological features of dura mater before and after uniaxial tensile tests. Particular attention was given to the three-dimensional arrangement of fibrous elements and spatial mutual relationship between collagen and elastic fibres. Because elastin elements were often obscured by dominating collagen fibres the authors made an attempt of better visualisation of elastin elements utilising selective fluorescent dye – sulforhodamine B.

2. Materials and methods

2.1. Materials

The research subject consisted of samples of the dura mater of the spinal cord. The material was harvested from the cadaver of 6 domestic half-year-old pigs (average weight 120 kg) obtained in a local slaughterhouse. In order to prepare appropriate specimens, the spinal cord had to be first extracted from the spinal canal in such a way dura mater remained intact. Then, the dura mater was gently separated from the surrounding tissues using a scalpel blade. Tests of mechanical properties were performed on post mortem specimens collected on the same day. All specimens were stored in the physiological saline solution until the performance of the measurement.

A special die-cutting tool was used to excise rectangular specimens from the collected dura mater, measuring 5 × 50 mm (width × length). The samples of the dura mater were excited in two directions: longitudinal (cranio-caudal) and circumferential (surrounding the spinal cord); altogether, 263 samples were excised. Specimens collected from the cervical (C), thoracic (Th), and lumbar (L) segments of the spine were analysed separately. The number of specimens subjected to mechanical testing with respect to the spine segments from which the specimen was collected (C, Th, L) as well as the direction of the specimen excision (longitudinal or circumferential) were presented in Table 1.

Table 1. Information about the number of specimens subjected to mechanical tests depending on the segment of the spine from which the specimen was collected (C, Th, L) as well as the direction of the specimen excision (longitudinal or circumferential)

Spinal segment	Sampling direction	Total amount
C	circumferential	30
	longitudinal	31
Th	circumferential	72
	longitudinal	60
L	circumferential	36
	longitudinal	34

2.2. Mechanical properties

Prepared specimens of the dura mater of the spinal cord were subjected to a uniaxial tensile test with the use of the MTS Synergie 100 testing machine. Specimens of the dura mater were placed in a special clamp, and additionally, they were glued to these clamps to further protect the specimens from slipping out. The initial distance between the clamps during each measurement was 10 mm, and the samples were stretched at a speed of 4 mm/min until they ruptured. Each actual measurement was preceded by initial cycles up to the displacement value of 1 mm to minimise the hysteresis effect's impact. The difference between the loading and unloading curves was getting smaller for subsequent cycles. It turned out that the beginning

of the fourth cycle coincided with the third cycle; therefore, the number of initial cycles was sufficient.

Based on the obtained force-displacement and stress–strain characteristics, the following parameters were determined: ultimate force (F_U), stiffness coefficient (k), ultimate tensile strength (σ_{UTS}), and Young's modulus (E). The E and k were determined in the linear range of characteristics (stress–strain, load-displacement) using a slope value. Before each measurement digital micrometer (Mitutoyo Digimatic IP65 0–25; 0.001 mm) was used to measure the thickness of the tested specimens (t_0). Then the current cross-sectional area A [mm^2] was calculated according to the formula, which takes into account the elongation of the sample during the conducted uniaxial tensile test [38] (1):

$$A = \frac{l_0 \cdot w_0 \cdot t_0}{\Delta l + l_0}, \tag{1}$$

where: l_0 [mm] is the original length of the specimen, w_0 [mm] is original width of the specimen, t_0 [mm] is original thickness of the specimen, Δl [mm] is elongation.

2.3. Statistical analysis assumptions

To determine if there are statistically significant differences among groups of an independent variable, a non-parametric Kruskal–Wallis and multiple comparison test were applied. It should be noted that some assumptions for ANOVA were not satisfied by measurement data. As a consequence, the test based on comparison of the mean ranks was chosen. The mentioned groups relate to the samples consisting of measurements corresponding to the respective segments of the spine, i.e., cervical (C), thoracic (Th), lumbar (L), in longitudinal and circumferential directions. Calculations were made with the use of StatSoft Statistica v. 12.1 software package. The null hypothesis was accepted with p-value level greater or equal to 0.05.

2.4. Morphology

Before and after the mechanical tests, the representative tissue samples were accessed morphologically. Specimens were fixed in 10% buffered formalin, dehydrated and paraffin-embedded, sectioned to 5 μm according to a standard protocol. These samples were then stained with hematoxylin and eosin (H&E) (Sigma-Aldrich, Munich, Germany) and analysed using light microscope Axio Imager.M1m (Carl Zeiss Microscopy GmbH, Jena, Germany) fitted with a DIC imaging facility.

Chosen samples were examined in their native state (without any fixation), and their morphological features were observed with a confocal microscope LSM 510 META (Carl Zeiss Microscopy GmbH, Jena, Germany; objective C-apochromat 40×/ 1.20 W Korr). The selective sulforhodamine B (SRB powder, Sigma-Aldrich) was used (after dissolving it in PBS to obtain 1 mg/ml solution), which visualised elastic elements even more efficiently. Samples were incubated for 5 min. with the SRB solution and then washed with PBS. The 561 nm laser was used to excite SRB and the corresponding signal from elastin was collected within a long pass LP 575 filter. Confocal pinhole was adjusted to 1AU. 8 bit 512 × 512 images were collected with pixel dwell of 1,61 μs. Earlier, the similar method has been utilised by us to visualise elastin elements in human *fascia lata* in a more efficient way [29].

3. Results

3.1. Mechanical properties

Mechanical testing of the spinal dura mater samples was performed under uniaxial loading and quasi-static conditions. Based on the collected data obtained during the measurements, the graphs of the dependencies were determined: force-displacement and stress–strain. The nonlinear stress–strain curves had a similar shape depicted in Fig. 1 were typical of soft tissues with three regions (toe, linear, yielding and failure). Then, the mechanical parameters, e.g., ultimate force (F_U), stiffness coefficient (k), ultimate tensile strength (σ_{UTS}), and Young's modulus (E), were determined, as described earlier by other authors [22], [39]. The completed mechanical tests have shown, above all, the anisotropic properties of the examined tissue. The results were summarised in column graphs to display mechanical parameters obtained for samples cut in different directions (longitudinally contra circumferential) more clearly (Figs. 2a–d).

The value of *ultimate force* (F_U) was higher for longitudinal samples when compared to circumferential samples for all examined spine regions, and all obtained differences were statistically significant for all examined spine levels. The highest ultimate force was obtained for longitudinal thoracic samples (amounted to 13.75 ± 5.14 N), and the lowest for circumferential

lumbar samples (amounted to 3.45 ± 1.80 N). The highest F_U for circumferential samples were obtained for cervical level (amounted to 8.10 ± 2.66 N) (Fig. 2a).

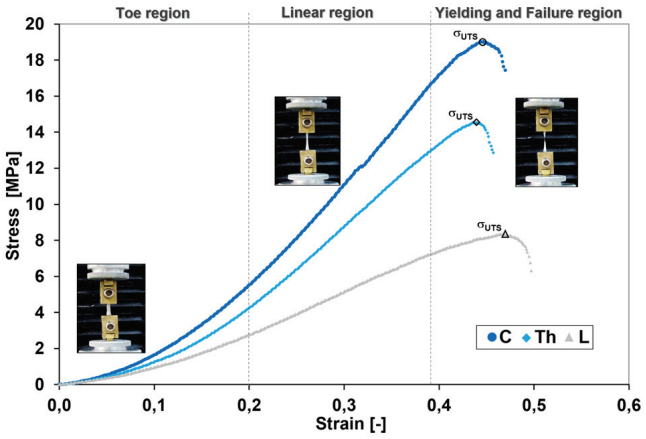

Fig. 1. The example of typical nonlinear stress–strain curves with three regions (toe, linear, yielding and failure) obtained for circumferential samples harvested from each spinal dura region: cervical (C), thoracic (Th), lumbar (L)

In general, the values of higher *stiffness coefficient* (k) were detected for longitudinal samples of spinal dura mater. The highest value was obtained for samples coming from thoracic level (amounted to 3.84 ± 1.56 N/mm). This value was two times greater when compared to circumferential samples harvested from analogical spinal level. Moreover, the most striking (even 3.5 times greater) and statistically significant differences of the stiffness coefficient values were noted between longitudinal and circumferential samples for lumbar level (with the lowest value amounted to 1.01 ± 0.6 N/mm). Pairwise comparison of circumferential and longitudinal samples did not show the significant differences only for cervical spine segment. The highest value of k regarding circumferential samples was obtained in the case of cervical spine (amounted to 2.57 ± 0.77 N/mm) (Fig. 2b).

To determine the ultimate tensile strength (σ_{UTS}), the *thickness* (t_0) of dura mater for samples obtained from all three spine regions was measured. It occurred that samples harvested from the cervical region were the thickest (0.138 ± 0.032 mm). The thickness of the dura mater samples coming from thoracic level amounted to 0.122 ± 0.026 mm and for lumbar level 0.106 ± 0.022 mm.

The highest value of *ultimate tensile strength* (σ_{UTS}) was obtained for longitudinal thoracic samples (amounted to 35.88 ± 10.90 MPa) and the lowest for circumferential lumbar samples (amounted to 10.03 ± 6.37 MPa). The most prominent directional differences (up to 3 folds) were observed for lumbar level. In the case of circumferential samples, the highest σ_{UTS} value was noted for lumbar level (amounted to 17.15 ± 6.54 MPa). For longitudinal samples, no statistically significant differences in ultimate tensile strength were noted regardless of the spine level (Fig. 2c).

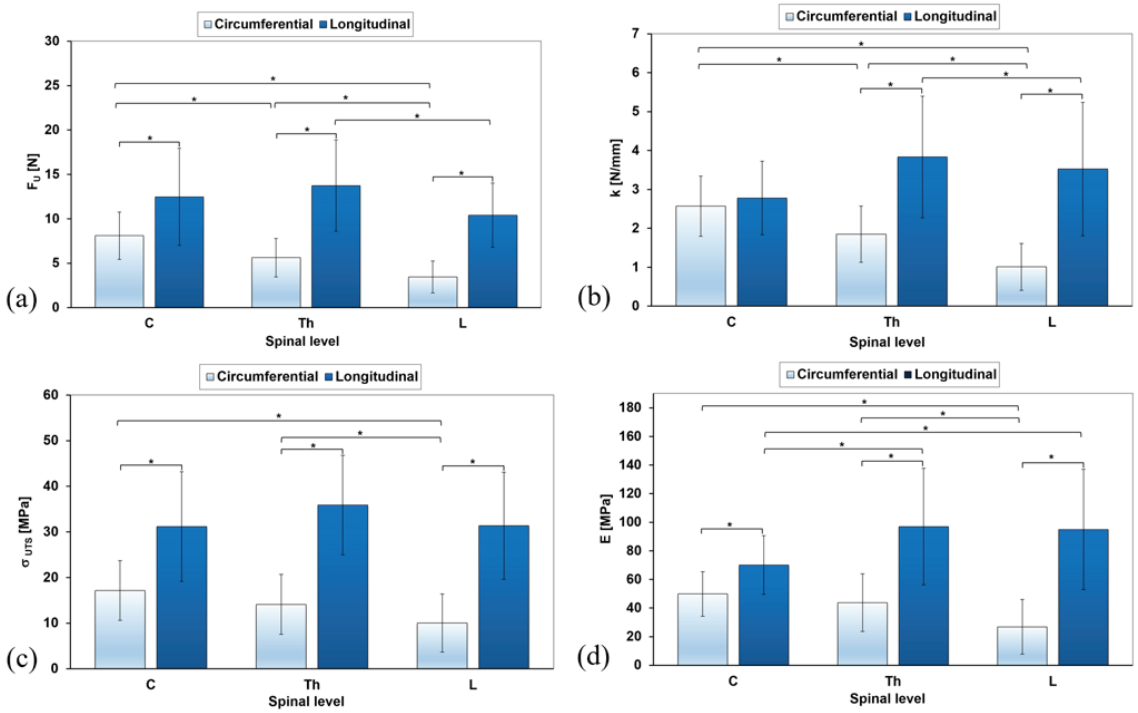

Fig. 2. The mean and standard deviation values for measurements of the: (a) ultimate force (F_U), (b) stiffness coefficient (k), (c) ultimate tensile strength (σ_{UTS}), and (d) Young's modulus (E); * – statistically significant difference between groups

The highest value of *Young's modulus's* (*E*) (96.97 ± 40.77 MPa) was detected for longitudinal samples harvested from thoracic spinal level. Additionally, the lowest Young's modulus (amounted to 26.85 ± 19.08 MPa) was noted for circumferential lumbar samples and this value was much lower (even more than 3.5 times) compared to longitudinal lumbar samples. The highest value of Young's modulus for circumferential samples was obtained for cervical level (amounted to 49.83 ± 15.50 MPa). Young's modulus for circumferential samples was significantly different in the case of cervical and lumbar levels and in the case of thoracic and lumbar levels. In contrast, statistically significant differences haven't been confirmed for cervical and thoracic levels. On the other hand, the only case in which statistically significant differences haven't been noticed for longitudinal samples was between thoracic and lumbar levels (Fig. 2d).

3.2. Morphological results

Dura mater samples originating from different spine levels have shown similar morphological features. Spinal dura under light and confocal microscopy was composed mainly of fibrous proteins, e.g., collagen and elastin fibres. Collagen fibres markedly prevailed over elastin fibres. They were organised in thick regular bundles running parallel to each other. Those bundles, on longitudinal sections, exhibited characteristic undulating appearance (crimped pattern) and were interconnected by much thinner, loosely and more randomly arranged collagen fibres (Fig. 3).

Because elastin elements were less numerous when compared to collagen fibres, their architecture was much more obscured. However, thanks to sulforhodamine B, a selective elastin stain, efficient visualisa-

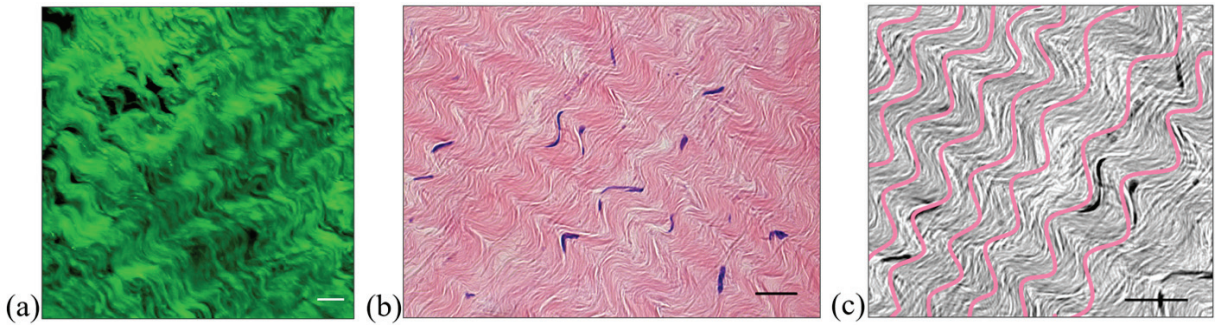

Fig. 3. Morphological features of dura mater: (a) confocal microscope image showing autofluorescence of collagen fibres in sample of cervical dura mater; collagen bundles run parallel to each other exhibiting characteristic undulation; (b) example of light microscopic image of lumbar dura mater with regular parallel bundles of collagen; H&E staining; (c) scheme showing characteristic, parallel, the undulating appearance of collagen bundles (crimped pattern). Scale bars = 20 μm

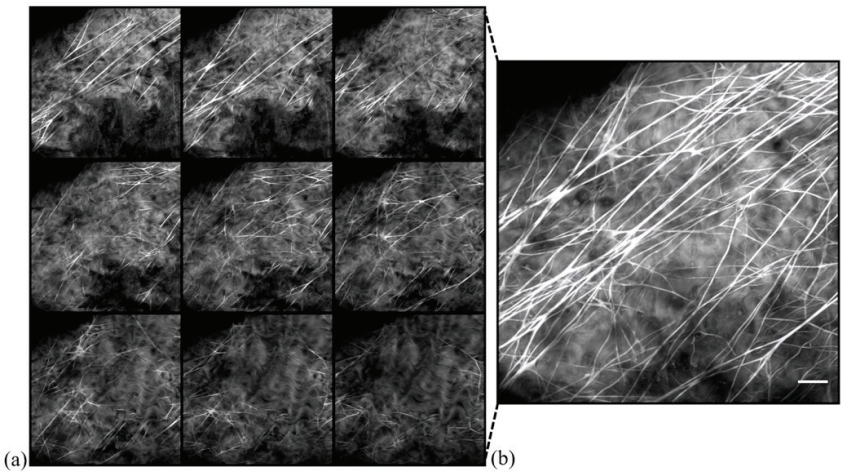

Fig. 4. Confocal microscope images of cervical dura mater selectively stained with SRB. (a) Series of sectional (distance of 1.5 μm in *Z*-axis between sections) images showing elastin fibres at multiple places throughout the sample's depth. (b) *Z*-stack of images obtained using the max intensity projection (covering approx. 14 μm in *Z*-axis); *z*-projection allowed to get more realistic 3D impressions of the elastic fibres network within the entire sample. Scale bar = 20 μm

tion of the elastin fibres network was possible. Elastin fibres were diverse in terms of thickness and length and exhibited more random distribution, running in different directions and forming a spatial network, presented at many examined tissue levels (Fig. 4).

Thick elastic fibres often followed the main direction of collagen bundles, sometimes "sewing them through" (Fig. 5).

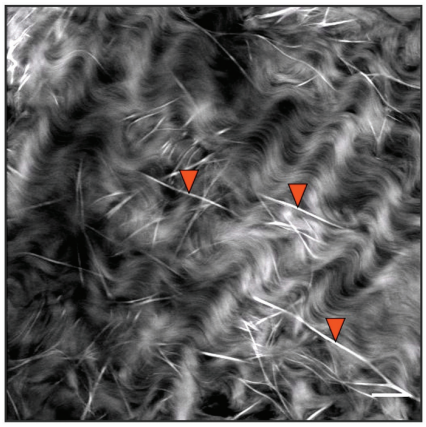

Fig. 5. Confocal microscope image of elastin fibres within the cervical dura mater; sample selectively stained with SRB; some of the thick elastin fibres (arrowheads) "sewing through" parallel arranged collagen bundles. Scale bar = 20 μm

An amorphous extracellular matrix occupied the space between the fibrous elements. The cellular component present within examined dura mater samples was relatively scarce. It was represented mainly by cells showing the morphological features of the fibroblastic cell population (spindle or stellate cells with centrally placed oval or round nucleus and scarce cytoplasm).

Before mechanical tests (Fig. 6a), the collagen bundles exhibited a distinct undulating appearance (crimped pattern). In contrast, in samples subjected to tensile force, the reduction of undulation was observed (as a consequence of stretching of collagen bundles along the alignment direction). Additionally, after uniaxial tests spaces between collagen bundles appeared to be wider (Fig. 6b). Upon stretching, both collagen bundles and fibroblasts morphology were subject to change. They have lost their plump, stellate appearance and become more elongated and spindle-shaped.

4. Discussion

Mechanical testing of the spinal cord, as well as the dura mater, are usually performed on animal specimens such as rats [15], dogs [21], sheep [26], [35], swine [4], [16], [28], and cattle [25]. It could be explained mainly by easier access and higher homogeneity of the animal material resulting from, among others, similar age and feeding methods of the individuals coming from a single breeder. Studies presenting the results of strength testing of the spinal dura mater performed on human specimens are much less numerous [5], [25], [34], [37]. In view of the above as well as the ethical issues and limited availability of human tissues, in the current study, the porcine dura mater has been used. Genetic, anatomical, histological and pathophysiological similarities make pigs the best animal model close to

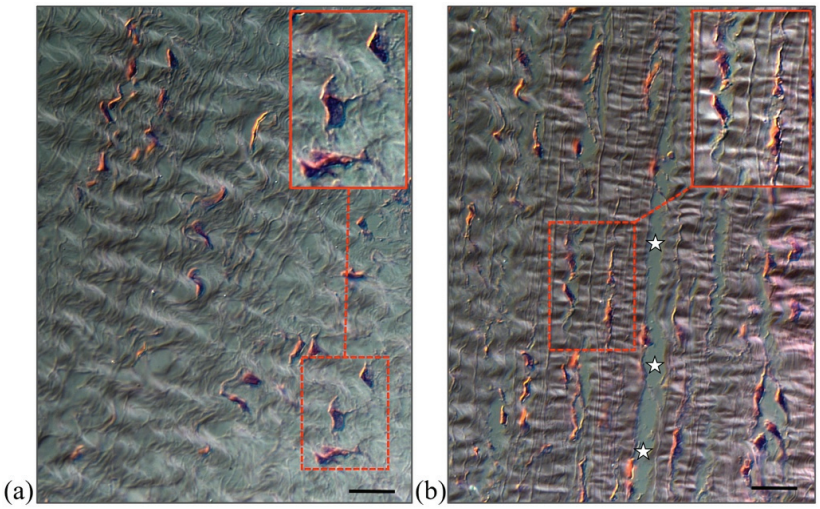

Fig. 6. The dura mater from the lumbar parts of the spinal cord before (a) and after (b) the mechanical test imaged with DIC. Under tension, groups of neighbouring, parallel aligned collagen bundles were separated by wider gaps (asterisks) compared to gaps presented in samples examined before mechanical tests. The insets show fibroblast; the difference in cell shape: before the mechanical test, fibroblasts were more plump or stellate and after, they exhibited a more elongated, spindled appearance. Scale bars = 20 μm

humans. For this reason, at present, swine material is often used in spine research [4], [11], [16], [22], [28]. Nevertheless, there is information in the literature mainly on the entire spinal cord of the pig [1], [14]. In contrast, information on the mechanical properties of the spinal dura mater of the pig is underrepresented.

Despite its apparently simple structure, dura mater is the most durable among all three meninges and consequently plays the protective role most effectively [27], [32]. Spinal dura mater occurred to be considerably stiffer than the spinal cord [21], [25]. It was shown that elastic modulus for spinal cord in tension and compression had a value of 1 MPa, while elastic modulus in tension for dura mater was 60 MPa. Disruption of the continuity of dura mater significantly reduces strength of the whole spinal cord. Even a small incision of the dura mater reduces the value of Young's modulus by more than 15 times (E = 1.40 MPa in the case of spinal cord with intact dura mater and E = 0.09 MPa in the case of the spinal cord without dura mater) [17]. All the above proves the dura's key mechanical function in protection of the central nervous system [15].

The mechanical behaviour of the spinal dura mater can be characterised by its strength parameters. As it was reported, these mechanical parameters differ depending on the level of the spine as well as the direction the dura mater specimens were excised (longitudinal versus circumferential) [4], [21], [25], [28]. Collagen bundles within spinal dura mater are oriented mostly parallel to the long axis of the vertebral column (in cranio-caudal direction). Consequently, in case of the spinal dura specimens excised longitudinally collagen fibres are arranged according to the direction of stretching (i.e., collagen fibres run mainly cranio-caudal with some little degree of obliquity), whereas in specimens excised circumferentially the fibres are arranged perpendicularly to the direction of loading [21], [25], [32]. As a result the greater tensile strength and stiffness of the dura mater has been observed for longitudinally excised samples, what was demonstrated for humans [21], dogs [18], pigs [28] and bovis [25].

The results of our study confirmed that there are significant differences of the values of mechanical parameters in the longitudinal versus circumferential direction Obtained directional differences provide clear indications of the anisotropic nature of the porcine dura mater.

Not only the stiffness and strength but also the maximum force required to rupture the dura mater of the spinal cord was dependent on the direction of the specimen excision. It was shown that in the case of longitudinal samples of human spinal dura maximum force was from 5 to 11.4 times greater than the maximum force needed to rupture the circumferential samples [21]. In another study, the force required to rupture the longitudinal samples harvested from lumbar dura mater was 3.5 to 6.7 times greater than circumferential samples [37]. According to some authors, the greatest directional differences exist in the case of the lumbar spine (the maximum force for lumbar longitudinal specimens was 3 times higher than for circumferential specimens) and the lowest in the case of the cervical spine (maximum force was 1.5 times greater for the cervical specimens excised longitudinally when compared with excised transversely) [21]. These results greatly correspond with results obtained by us, whereby in our study, the most pronounced directional differences were observed for lumbar region (F_U was 3 times greater for specimens excised longitudinally compared to those excised transversely).

Also, the stiffness of the longitudinal dura samples was much higher when compared with the stiffness of circumferential samples as it was found in case of human spinal dura mater [21]. Our study proved the same correlation for porcine dura mater. Analogical results were obtained for pigs by Cavalier [4], who confirmed that spinal dura stiffness was greater in the longitudinal direction ranging from 1.28 to 5.32 N/mm for each spinal level).

One of the integral elements of the mechanical tests, particularly determination of the tensile strength, is measuring the thickness of the examined tissue. The thickness of the dura mater varies between species (for example, for humans it ranged from 0.350 to 0.249 mm [10] and for sheep from 0.268 to 0.103 mm [35]). In the case of pigs, in our study, the thickness of the spinal dura ranged from 0.138 to 0.106 mm, while, according to Cavalier, it ranged from 0.117 to 0.053 mm [4]. Moreover, spinal dura thickness varies depending on the spine level. Our results showed that the thickness of the porcine spinal dura mater decreased gradually from head to tail. Similar observations were made for other animal species [15], [21], [35]. Interestingly, it was shown that the thickness of human spinal dura was highest at thoracic and lowest at lumbar level and was found to increase slightly with age being similar between men and women [10].

In our study, similarly to studies conducted by other authors on pigs, humans and sheep, tensile strength for longitudinal samples for all spine segments was significantly higher than for the circumferential samples [4], [25], [35]. The ultimate tensile strength values obtained by us for circumferential samples decreased gradually from head to tail. The same tendency was observed by other authors [4], [28]. It should be un-

derlined, however, that values of the ultimate tensile strength obtained by Sudres were more than 3 times lower compared to ours. Moreover, ultimate tensile strength obtained by us for porcine longitudinal specimens were greater (amounted to approx. 31–36 MPa) than the values obtained for the human spinal dura mater (amounted to approx. 8–20 MPa) [25].

According to some authors, there is no significant difference in elastic moduli values for longitudinal and circumferential spinal dura samples harvested from the cervical level [35]. Others, including us, confirmed that the values of Young's modulus occurred to be higher for longitudinal samples when compared to circumferential at all spinal levels [4]. Values of Young's modulus for longitudinal samples we obtained for pigs accounted for approximately 70 to 97 MPa, while in studies conducted by Cavalier this value ranged from approximately 117 to 250 MPa [4] and by Sudres accounted for approximately 22 to 38 MPa [28]. Analogical values for longitudinal samples in human's lumbar varied between 65 and 102 MPa for fresh samples [25]. For porcine circumferential samples the Young's Modulus obtained by us (approximately 27–50 MPa) was lower when compared to analogical value obtained by Cavalier (approximately 54–86 MPa) [4] but higher when compared to Sudres (approximately 12–21 MPa) [28]. For comparison the value of Young's Modulus for human's lumbar circumferential spinal dura mater accounted for approximately 4–6 MPa [25], and for ovine spinal dura (approximately 8–16 MPa in the longitudinal direction and approximately 3.5–14 MPa in the circumferential direction) [35].

Unfortunately, after analysing the above presented strength parameters, apparent discrepancies in results obtained by different authors could be observed. There are many possible causes of these discrepancies, such as lack of an agreed test procedure, different grips and fixing methods, different stretching speeds, performance of strength tests until rupture or acquisition of a particular strain value, different donors and different conditions of storage of the material. Particularly the latter, to a large degree, influences the obtained stress values (specimens not stored in saline solution showed lower strength values) [25]. In addition, it is known that storage of human dura mater in glycerol has little effect on the mechanical strength and storage in saline solution up to 30 days (after storage in glycerol) does not affect the mechanical properties if sterility is maintained [34]. For this reason, during our study, we have used fresh, unpreserved tissue (dampen samples with a small amount of saline solution to protect them from drying). In the case of *in vitro* studies, the research results can also be largely influenced by the time differences between the performance of the test and the death of the individual from whom the spinal dura mater was collected. The significant effect of time on the determined mechanical properties of the spinal cord was presented in studies carried out by Oakland and Wilcox [20].

At present, available data concerning structural properties of the dura mater apply to a great extent to the cranial dura mater, while characteristics of the spinal dura mater have been less explored. For this reason, precise morphology of spinal dura remains inconsistent. Older studies have shown that spinal dural collagen fibres are not preferentially aligned in the longitudinal direction and that a substantial part of the total collagen fibre has been observed to be oriented in the circumferential direction [8], [24]. Then, it was shown that dura mater is a well-defined 2 or 3-layered structure [23] with parallel overlapping layers of collagen in which the orientation of these layers changes at different depths [33]. Then, it was reported that spinal dura is multilayer tissue consisting of sheets of tightly packed collagen fibres oriented mainly parallel (longitudinally), intermixed with elastin fibres exhibiting more random distribution [18], [25]. In general, our observations are consistent with these results. The spinal dura mater was a fibrous connective tissue rich in collagen and elastin fibres embedded in an intercellular matrix. Thanks to collagen fibres, spinal dura mater exhibits its tensile strength, and thanks to elastin, elasticity. Collagen fibres within the spinal dura were preferentially aligned in the longitudinal direction and were interspersed with elastic fibres.

The concentration and direction of elastin fibre, besides elasticity, also influences the stability and tensile properties of the dura, as well as its ability to resist mechanical loads in flexion and extension [7]. According to some research, the density of collagen and elastin fibres rises from inner to outer direction [5], [18]. In our study, both the density and orientation of the fibres was dependent on the depth of the sample, but the collagen architecture was much more regular compared to the elastin one. In general, collagen fibres and thick (mainly straight and running parallel) elastin fibres were visible under the light microscopy (as observed by Nomarski differential interference contrast microscopy). On the other hand, visualisation of thinner and shorter elastic fibres, particularly accessing their three-dimensional arrangement is much more demanding. The reason is that elastic fibres are markedly outnumbered by collagen fibres and, what is more, collagen fibres are tightly packed obscuring elastin components. Usually to show their morphology

more clearly the special histological staining methods (for example Weigert, Verhoeff Van Gieson), but these methods are destructive and incompatible with intravital studies. Additionally, noninvasive imaging of elastic structures can be best achieved by second harmonic generation (SHG) signals, still, this technique is expensive, technically demanding and not entirely specific (tissue components, like actin, yield SHG signals as well) [2]. The staining of elastin by fluorescent labelling using sulforhodamine B (SRB) assay provided not only a clearer picture of the elastin network, but could be displayed in its native state, i.e., without a need of sample fixation. To the authors' best knowledge, it is the first time of labelling this important fibrous protein within dura mater using SRB. This method was fast, relatively inexpensive and technically simple to perform. The obtained images were processed with ImageJ software which allowed them to learn more about the three-dimensional arrangement of elastin fibres and to characterize their network in full thickness dura samples.

Spinal dura mater samples subjected to uniaxial tests exhibited non-linear stress–strain response similar to other collagenous, load bearing soft tissues (e.g., ligaments, *fascia lata*, normal and aneurysmal arterial tissues, denticulate ligaments) [12], [13], [22], [31]. Under the influence of uniaxial tensile loading, collagen bundles begin to orient themselves in the direction of loading, straighten, and stretch. That is why the native crimped pattern of collagen fibres, present at rest, disappeared. It is suspected that this crimped configuration acts as a buffer against fibre damage and reappears (within the limit of linear region) only when the load stops and the stretched collagen bundles go back to the resting state, thanks to the elastin fibres [6].

The dura mater has the same embryological origin as the fascial system [3]. Despite no comprehensive "fascia" definition for a long time, recently, according to the newest classification of the fascial system, fascia is any tissue exhibiting features capable of responding to mechanical stimuli. As such, structures like, e.g., epineurium, epimysium, ligaments, tendons, aponeurosis and meninges, including dura mater, should also be considered fascia [3]. Loss of characteristic undulation (loss of crimped pattern) of collagen fibres as well as widening of the spaces between collagen bundles observed in case of spinal dura samples subjected to tensile strength, was very similar to pictures obtained by us in case of *fascia lata* [31]. Dura mater should be considered part of the widespread fascial body system [30]. In our opinion, it is another (indirect) proof that dura mater belongs to the fasciae.

5. Conclusions

Conducted mechanical tests confirmed viscoelastic properties of spinal dura mater. On the other hand, morphological studies demonstrated architectural evidence supporting the anisotropic behaviour of the examined tissue. Both, mechanical behaviour as well as structural features of the spinal dura mater, were analogous to fascial tissues, which constitutes indirect proof that the dura mater belongs to the widespread fascial system of the body. The study shows that the spinal dura mater regarding mechanical properties is not uniform along the entire length of the spinal cord. Values of mechanical parameters depend on the place (segment) and direction (longitudinal or transverse) of sample collection, while in the case of morphological studies, no significant differences were noticed. Tissue material collected from pigs seems to be a convenient and reliable animal model for spinal dura mater studies in humans.

The utilisation of fluorescent dye – sulforhodamine B appeared to be a non-destructive, fast, and efficient tool for visualising even the smallest elastic fibres on different depths of examined samples. To our knowledge, this study is the first attempt to use the SRB to determine elastin in the spinal dura mater. The obtained results may facilitate the creation of mathematical modes of dura mater, could be useful in evaluating and creating dura mater allografts, and predict the behaviour of this fibrous membrane during physiological or traumatic motions.

Conflict of interest

The author declares that there is no conflict of interest regarding the presented study.

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