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Tensile Mechanical Properties of the Cervical, Thoracic and Lumbar Porcine Spinal Meninges

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Abstract:

Background. The spinal meninges play a mechanical protective role for the spinal cord. Better knowledge of the mechanical behavior of these tissues wrapping the cord is required to accurately model the stress and strain fields of the spinal cord during physiological or traumatic motions. Then, the mechanical properties of meninges along the spinal canal are not well documented. The aim of this study was to quantify the elastic meningeal mechanical properties along the porcine spinal cord in both the longitudinal direction and in the circumferential directions for the dura-arachnoid maters complex (DAC) and solely in the longitudinal direction for the pia mater. This analysis was completed in providing a range of isotropic hyperelastic coefficients to take into account the toe region.

Methods. Six complete spines (C0 – L5) were harvested from pigs (2-3 months) weighing 43 +/- 13 kg. The mechanical tests were performed within 12h *post mortem*. A preload of 0.5N was applied to the pia mater and of 2N to the DAC samples, followed by 30 preconditioning cycles. Specimens were then loaded to failure at the same strain rate 0.2 mm/s (approximately 0.02/s, traction velocity/length of the sample) up to 12 mm of displacement.

Results. The following mean values were proposed for the elastic moduli of the spinal meninges. Longitudinal DAC elastic moduli: 22.4 MPa in cervical, 38.1 MPa in thoracic and 36.6 MPa in lumbar spinal levels; circumferential DAC elastic moduli: 20.6 MPa in cervical, 21.2 MPa in thoracic and 12.2 MPa in lumbar spinal levels; and longitudinal pia mater elastic moduli : 18.4 MPa in cervical, 17.2 MPa in thoracic and 19.6 MPa in lumbar spinal levels.

Discussion. The variety of mechanical properties of the spinal meninges suggests that it cannot be regarded as a homogenous structure along the whole length of the spinal cord.

51 **Abbreviations**

52 DAC: dura-arachnoid mater complex

53 PM: pia mater

54 ROI: region of interest

55 DIC: digital image correlation

56 CSA: cross-sectional area

57 GUM: Guide to the expression of Uncertainty in Measurement

58 SE : standard error

59 SD: standard deviation

60 RMSE (Root Mean Square Error).

61 ROM: range of motion

62 CSF: cerebrospinal fluid

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1. Introduction

The spinal meninges composed of the dura mater, the arachnoid mater and the pia mater, play a mechanical protective role for the spinal cord (Sakka et al. 2016). The dura mater is a fibrous, white, thick, and resistant membrane formed by a dense connective tissue poorly vascularized with a ratio of collagen I fibers to elastin fibers varying radially. The arachnoid mater can be described as an outer layer, consisting of a thin transparent membrane mainly composed of collagen and elastin fibers, attached to the dura mater by thin strands of collagen. The pia mater is mainly composed of predominantly longitudinally orientated collagen fibers, which carry larger branches of the spinal vasculature (Reina et al. 1997, 2020). As any biological soft tissue, meningeal mechanical properties are altered *post mortem* due to drying and the interruption of blood supply, thus leading to tissue degeneration (Garo et al. 2007, Fountoulakis et al. 2001). In consequence, the experimental testing methodology needs an thorough management of time and manipulation to provide reliable mechanical measurements in order to highlight the specificities of such biological soft tissues. Better knowledge of the mechanical behavior of these tissues wrapping the cord is required to accurately model the stress and strain fields of the spinal cord during physiological or traumatic motions (Bertram and Heil 2016, Fradet et al. 2016, Sparrey and Keaveny 2009, Kimpara et al. 2006, Khuyagbaatar et al. 2014). Such modeling tools allowed the correlation of the stresses fields in the spinal cord with clinical analyses (Lévy et al. 2020, Czyż et al. 2012), and the assessment of the effect of a specific surgery on these tissues (Heno et al. 2016).

The spinal dura mater was mainly tested in tension at different locations of the spine. A porcine cervical cartography of the mechanical properties showed differences between the dorsal and the ventral side of the dura mater (Mazgajczyk et al. 2012). A significant variability of properties was found between species, such as rats (Maikos et al. 2008), dogs (Patin et al. 1993), sheeps (Shetye et al. 2014), swines (Mazgajczyk et al. 2012), cattle (Runza et al. 1999) and humans (Runza et al. 1999, Chauvet et al. 2010). Due to ethical and logistical complications related to human species, the high similarity of porcine model to the human species on the genetic, anatomical, physiological, pathophysiological [Schomberg et al. 2017], and biomechanical [Wilke et al. 2011] levels, the swine model appears as the best transversal human models, next to non-human primates [Schomberg et al. 2017], and is increasingly used in spine research [Kim et al. 2019, Ramo et al. 2018b, Brummund et al. 2017, Fradet et al. 2016, Swindel et al. 2013]. In addition, similarities between human and porcine models were highlighted histologically for the dura mater (3 dural layers structure) [Kinaci et al. 2020].

An anisotropic behavior (in longitudinal or cranio-caudal direction and in circumferential direction) was also reported in most of the species except for the dog. One biaxial study confirmed this difference between these two mechanical orientations on ovine samples (Shetye et al. 2014). The anisotropic behavior was corroborated by histological studies describing, in particular, the longitudinal direction of fibers along the cord (Maikos et al. 2008, Chauvet et al. 2010). Among these studies, the spinal arachnoid mater was considered as a part of the spinal dura mater, excepted for two of them which assumed its adherence to the spinal pia mater (Fabris et al. 2019, Ramo et al. 2018c). The spinal arachnoid-dura tissue

101 continuity assumption was validated by others histological quantification, showing the arachnoid mater to
102 be the inner layer of the dura mater. The relative thickness of dura-to-arachnoid mater however suggested
103 a limited role of the arachnoid to the mechanical properties of the dura mater (Vandenabeele et al . 1996,
104 Reina et al. 1997). Then, quantification of the mechanical properties of pia and dura-arachnoid mater
105 complex (DAC) mechanical properties is not fully described along the whole spinal cord in the literature.
106 One study compared biomechanical properties of the ovine dura mater at six spinal levels (C₆, C₇, T₁₁, T₁₂,
107 L₄, and L₅). No significant difference of elastic moduli values between longitudinal and circumferential
108 directions at the cervical levels were notified, but this study showed that the ratio between the
109 longitudinal and circumferential elastic moduli were significantly smaller at the cervical and thoracic levels
110 (Yang et al. 2019). In addition, Mazgajczyk et al. (2012) highlighted a significative variability of DAC
111 mechanical properties along the porcine cervical segment between the dorsal and the ventral sides on
112 porcine models. Our hypothesis is that the elastic mechanical properties, which vary between the ventral
113 and dorsal sides, could also vary along the whole longitudinal spinal cord from the spinal cervical to the
114 spinal lumbar levels.

115 The effect of conservation methods on mechanical characteristics is not systematically quantified.
116 Freezing method at -4°C was shown to modify the mechanical properties variability of the human dura
117 mater through time, while the mechanical properties of the bovine dura mater decreases independently of
118 the time (Runza et al. 1999).

119 Concerning the spinal pia mater, two studies provided indirect measurements of the cervical
120 compressive and tensile mechanical properties on rabbits (Ozawa et al. 2004) and on human spinal cord
121 (Mazuchowski and Thibault 2003). More recently, quasi-static tensile tests and a dynamics mechanical
122 analysis were performed on cervical ewe pia mater assumed as an integrally laminated structure
123 composed of the arachnoid and the pia maters. This study highlighted the non-linear viscoelasticity of the
124 ewe pia-arachnoid structure (Ramo et al. 2018c).

125 The aim of this study is to quantify the elastic meningeal mechanical properties along the porcine
126 spinal cord in the longitudinal (cranio-caudal) direction and in the circumferential (surrounding the spinal
127 cord) directions for the DAC, and solely in longitudinal direction for the PM. A quasi-static loading close to
128 physiological solicitations was considered. In addition, the effect of the conservation method effect was
129 assessed by comparing flash frozen (at -80°C) (Sparrey et al. 2009) (different from a classical freezing at -
130 4°C) and fresh samples properties.

2. Material & Methods

2.1. Samples preparation

Two tissue structures were characterized in this study: the dura-arachnoid mater complex (DAC) and the pia mater (PM). Six complete spines (C0 – L5) were harvested from pigs (race: crossbreeding Yorkshire x Landrace, age: 2-3 months, weight: 43 +/- 13 kg, gender: 4 males and 2 females) one to two hours following euthanasia. The animals were euthanatized for reasons unrelated to this study. The spine was firstly extracted by a dissection on the dorsal side using scalpels and oscillating saw. This step was performed less than 35 minutes after euthanasia. The spine was then sectioned in three levels: cervical level (from the foramen magnum to the T1-T2 joint), thoracic level (from the T1-T2 joint to the T13-T14 joint, and lumbar level (from the T13-T14 joint to the L6-S1 joint). The spinal cord-meningeal complex, including the DAC, was carefully removed from the spinal canal through fine dissection with scalpels and gouge clamps, and small clamps (duration of 1 hour). The nerve roots and the denticulate ligaments were resected along the DAC. The spinal cord-meningeal complex from two spinal levels over three were stored in PBS at 4°C. The DAC and PM samples were prepared and tested within a 12 hours and 30 minutes after euthanasia. The remaining one third of spinal cord-meningeal complex was saved using flash-freezing (FF) method at -80°C [Sparrey et al. 2009] and thawed at 4°C and prepared and tested at 20°C. For all samples, the DAC was longitudinally incised on the lateral side of the spinal cord-meningeal complex and was removed from the cord. The same operation was done for the PM, which was also taken apart from the white and grey matters. All the fresh and FF samples were stored in PBS at 20°C to keep them moist after preparation and tested at room laboratory temperature of 20°C. The samples were placed between two pieces of sandpaper into which a window was cut, and a stochastic pattern of dots was applied using an oil-based black paint spray (Ptouch 2x + sspr 6pk flat black, Rust-Oleum Corporation, USA), (Fig 1 – A). This step was used to measure the length and the width of the sample as well as to perform strain and stress fields analyses. The support was then inserted and fastened into the clamps of the testing machine (GRIP ASST, T/C, 3200, Bose Corporation, Framingham, Massachusetts, US). The lateral side of the sandpaper support was cut before testing to allow traction with the clamps (Fig 1 – B). Two different orientations of clamping were used, leading to 2 different loading directions for the DAC: longitudinal and circumferential. PM samples were only tested in the longitudinal direction.

The length (L_0) of the samples was defined as the minimum distance between the clamps at the initial position before testing. The width (w) was defined as the lateral distance of the clamped sample at the initial time position before the mechanical test. Samples not fully attached samples to the clamps [Fig. C.4] or with a visible deterioration after attachment were excluded from the study. The reference thicknesses (t) of the DAC and PM were measured at 3 spinal levels (cervical, thoracic and lumbar) on an additional porcine subject to avoid damaging the samples due to supplementary manipulations. The DAC and PM samples were sectioned to a width of 1mm and a length corresponding to the longitudinal distance between two nerves roots. They were positioned on a black 120g paper. The samples' side with the largest area were glued on with the black paper by a

169 transparent agar substrate. Then, the samples were positioned on the edge on a graph paper. A Micro
170 C110 camera (Vision Research, 8GB) with a 105 macro lens (Nikon F2.8), as well as two light systems
171 (LED EFFILUX) were used to acquire high resolution images of the samples' thickness. Three samples
172 were sectioned in three sub-samples at each spinal level as defined in the first paragraph of this
173 section. Calibration of the camera was performed using three measurements of the graph paper. Ten
174 measurements were performed on each sub-sample. A total of 30 measurements by tissue (DAC and
175 PM) and by spinal level (cervical, thoracic and lumbar) were performed. The cross-section area (CSA)
176 of the sample was assumed to be rectangular and defined as the product of the width (w) by the
177 thickness (t).

178 **2.2. Tensile Testing**

179 Both types of sample were submitted to the same testing protocol with specific preload and
180 threshold values for the pre-cycling phase. A preload of 0.5 N was applied for the pia mater and of 2 N
181 for the DAC samples followed by 30 preconditioning cycles (frequency varies with the time to reach
182 the preload, the magnitude: 1 N). They were performed to compensate the retraction of the sample
183 and remove any buckling of the sample before testing. The amplitude of preloading was based in the
184 literature [Shetye et al. 2014, Ramo et al. 2018a,b,c]. A moderate tensile ramp at 0.2 mm/s was
185 chosen in accordance with *in vivo* experiment of porcine spinal-cord-pia-arachnoid construct [Ramo et
186 al. 2018b] and reported by the literature for *ex vivo* ovine dura mater and pia-arachnoid complex
187 [Ramo et al. 2018a, c].

188 The tests were stopped at failure or when the maximum displacement was reached (Fig. 2,
189 TableB.1. in Appendix B).

190 **2.3. Data acquisition**

191 A testing machine Bose© ElectroForce 3200 Series (Bose Corporation, Framingham,
192 Massachusetts, US) was used to perform tensile tests (Fig.1). The loads data were acquired with a 22 N
193 load cell (reliability manufacturer error of 0.11 % and a repeatability error of 0.19 %) at a sampling
194 rate of 100 Hz. Dimensions (length and width) as well as the displacements of the samples.
195 Complementary displacement and strain fields were measured by 2D digital image correlation (DIC)
196 (ARAMIS 5 M, GOM mbH, Germany) at 15 frames per second.

197 **2.4. Data analysis**

198 *Linear elasticity*

199 The force/displacement curves were zeroed for comparison by subtracting the minimum
200 measured displacement to the initial measured displacement. Each engineering stress-strain curve
201 was computed from the measured force/displacement curves as follows:

$$202 \quad \sigma = F/CSA \quad \text{Eq. (1)}$$

$$203 \quad CSA = t * w \quad \text{Eq. (2)}$$

$$204 \quad \varepsilon = \Delta L/L_0 \quad \text{Eq. (3)}$$

205 where equation (1) defines the engineering stress (σ) as the ratio of the tensile force (F) over the
 206 initial the cross-sectional area (CSA) on which the force is applied. The initial cross-sectional area
 207 (Eq.2) of the sample is the product of its thickness (t) by its width (w) in his undeformed state.
 208 Equation (3) allows computing the engineering strain (ε) as the ratio of the measured displacement
 209 (ΔL) over the initial length (L_0) of the sample.

210 Three regions of interest (ROI) were identified on force/displacement curves to performed the
 211 post-processing of the stress/strain curves. The toe region (ROI 1 in Fig.3) corresponds to a non-linear
 212 region before the linear region (region 2 in Fig.3). The elastic limit is defined as the maximum stress
 213 point in the linear region. The third region follows the elastic limit and corresponds to a non-linear
 214 region called damage region (ROI 2 in Fig.3). The beginning of the damage region was defined by the
 215 first observed macroscopic drop in the force-displacement curve even if fiber ruptures could be
 216 present before this non-linear ROI. Several others local damage sub-regions describing a progressive,
 217 in stages, load loss linked with the progressive in stages rupture. To determine the range of the linear
 218 region, the second derivative of each force/displacement curve was computed and the maximum and
 219 the minimum inflection points were identified. The maximum and the minimum values of the second
 220 derivative were found and projected on the force/displacement curves to establish the elasticity
 221 region by two points corresponding to the stress/strain curve as follows. The toe stress (σ_{toe}) and
 222 strain (ε_{toe}) represent the values of the first elastic limit point and the damage stress (σ_{damage}) and
 223 strain (ε_{damage}) represent the values of the second elastic limit point (Fig. 3). A linear regression was
 224 computed between the elastic limits ($R^2 > 0.99$ for 111 over 119 fresh samples, and 21 over 22 flash
 225 freezing samples). The elastic moduli were computed from the linear regression defining equations
 226 (1), (2) and (3). A comparison of elastic moduli between the fresh and flash-frozen samples was
 227 performed only for the thoracic spinal samples. This choice was made to avoid the inter-level spinal
 228 variability of the samples.

229 *Hyperelasticity*

230 A isotropic hyperelastic one-degree Ogden model was fitted on each experimental curve depicted
 231 on Fig. 4. The uniaxial stress-stretch Ogden function is expressed as :

$$232 \quad \sigma = \frac{2\mu}{b} \left(\lambda^{b-1} + \lambda^{\frac{-b}{2}-1} \right) \quad \text{Eq.4}$$

233 Where μ is the shear modulus and b is a material constant and the stretch ratio defined as $\lambda = \varepsilon + 1$.
 234 The curve fitting was performed with the nonlinear least squares method using the fit function in
 235 Matlab R2018b (MatWorks, Natick, MA, USA) and evaluated evaluated by a R^2 and RMSE (Root Mean
 236 Square Error).

237 **2.5. Statistical analysis**

238 The uncertainty of samples' CSA, and its effect on the linear modulus, due to the use of a sample
 239 thickness estimated from a different animal, was evaluated. This uncertainty evaluation was based on
 240 the ISO 98:1995 Guide to the Expression of Uncertainty in Measurement (GUM) (ISO 98:1995 GUM)
 241 and used the Monte-Carlo simulation approach described in the GUM supplement 1 (JCGM 101:2008).

242 The model of surface uncertainty is defined as presented in equation (4), where u_s is the uncertainty
243 of the surface value, $u_{w_{rep}}$ is the uncertainty induced by the repeatability of width measurements,
244 $u_{w_{resolution}}$ is the uncertainty induced by the DIC resolution to measure the width, and $u_{th_{rep}}$ is the
245 uncertainty induced by the repeatability of thickness measurement.

$$246 \quad \quad \quad 247 \quad \quad \quad u_s = \left(u_{w_{rep}} + u_{w_{resolution}} \right) * u_{th_{rep}} \quad \quad \quad \text{Eq.(4)}$$

248
249 An algorithm (Solaguren-Beascoa Fernandez et al. 2009) was used with the following input
250 distributions:

- 251 • $u_{w_{rep}}$ was defined as a normal distribution from the mean of and the standard deviation (SD)
252 of $w_{measures}$.
- 253 • $u_{w_{resolution}}$ was defined as a uniform distribution from $-DIC_{resolution}$ to $+DIC_{resolution}$.
- 254 • $u_{th_{rep}}$ was defined as a normal distribution from the mean of and the standard deviation
255 (SD) of $th_{measures}$.

256
257 $w_{measures}$ represents all the width measurements by type of tissue and by spinal level, $w_{resolution}$
258 represents the resolution of the DIC, and th_{rep} represents all the thickness measurements by type of
259 tissue and by spinal level.

260 Then, a probabilistic range of linear modulus is provided based on the 32th and the 68th percentile
261 values of the measurement CSA distribution for each sample, corresponding to the most likely physical
262 confidence interval. Then the 32th and the 68th probabilistic value of elastic modulus were calculated
263 from the 32th and the 68th probabilistic value of the CSA. The Monte-Carlo GUM methodology is
264 described in [appendix A](#).

265 All data were statistically analyzed as follows. The normality of each distribution was verified
266 using Shapiro-Wilk normality tests. Due to the heterogeneity of normal distribution and the unequal
267 distribution size, Wilcoxon rank sum test was performed to quantify the significant difference of all
268 variables (force, stress, displacement, strain, elastic modulus) with respect to the spinal levels, the
269 orientation of loading for the DAC as well as the conservation methods. The significance level was set
270 to $p < 0.05$. Data analysis was performed using Matlab software (R2018a version,
271 Matlab®MathWorks,1984). Additionally, Bayesian linear mixed models (blme package -
272 <https://github.com/vdorie/blme>) were fitted with R [R Core Team 2018] to assess the dependency of
273 our result to the subjects and computed the standard errors (SE) of the elastic modulus taking into
274 account the between-subjects and within-subjects variabilities [Tirrell et al. 2018].

275 **3. Results**

276 The description of fresh and flash-frozen samples which were tested, excluded and analyzed is
277 summarized in [Table 1](#). The exclusion criteria are the following: sample damaged before being
278 fastened into the clamps, sample not well fastened in the clamps, issue due to an operator error with
279 the control command of the computer. Ten fresh samples (0 from longitudinal DAC, 6 from
280 circumferential DAC, 4 from longitudinal pia mater) and 3 flash-frozen samples (0 from longitudinal
281 DAC, 2 from circumferential DAC, 1 from longitudinal pia mater) tore at the middle of their length. All
282 experimental tests were completed less than 12 hours and 30 min after euthanasia of the animal
283 (mean: 7 h 26 min; max: 12 h 23 min).

284 The dimension of samples from the experimental measured are depicted in [Table 2](#). The samples'
285 range of length is between 8.33 mm and 15.49 mm for the longitudinal DAC while it is between 6.36
286 mm and 18.51 mm for the circumferential DAC. The range of length is between 6.23 mm and 13.91
287 mm for the longitudinal PM samples. The DAC sample harvested at the thoracic level are the thickest
288 (0.28 mm) while it is the cervical level for the PM (0.22 mm). The sample's CSA is the greatest at the
289 thoracic levels for the longitudinal and circumferential DAC and the longitudinal PM (with a mean
290 value of 3.77 mm², 4.84 mm² and 2.44 mm² respectively).

291 [Figure 4](#) reports the corridor of mechanical properties of the fresh samples: the DAC and the PM
292 behavior in longitudinal direction and the DAC behavior in the circumferential direction. The lower and
293 upper curves describe the experimental corridor (shaded area) defining the range from minimum and
294 maximum engineering stress/strain curves. Solid curves represent the typical stress/strain curve.

295 The elastic modulus of DAC in the longitudinal direction was found to be significantly different
296 between the cervical level (22.35 ± 10.0 MPa) and the two others levels, thoracic (38.1 ± 12.6 MPa)
297 and lumbar (36.6 ± 12.6 MPa). The elastic limit or damage stress/strain point of the cervical samples
298 (0.25 ± 0.09) is 1.7 times superior when compared to the thoracic samples (0.15 ± 0.09), and 1.4 times
299 inferior than the lumbar samples (0.18 ± 0.09). The elastic limit (damage strain) of the circumferential
300 lumbar samples (0.32 ± 0.14) is 1.5 times superior than for the thoracic samples (0.21 ± 0.08) and 1.4
301 times superior than the cervical samples (0.23 ± 0.1). Elastic modulus of the thoracic samples ($21.2 \pm$
302 3.0 MPa) is 1.7 times superior than lumbar samples (12.2 ± 4.4 MPa) and slightly superior than cervical
303 samples (20.6 ± 8.1 MPa). Significant differences were found between longitudinal and circumferential
304 elastic moduli between the thoracic (38.1 ± 12.6 MPa against 21.2 ± 3.0 MPa respectively) and lumbar
305 spine (36.6 ± 12.6 MPa against 12.2 ± 4.4 respectively) samples, but not with the cervical samples
306 ([Table 3](#)). No significant difference was found between the fresh samples and the flash frozen samples
307 for the DAC ([Table 4](#)).

308 No significant difference was noticed between the levels for the elastic modulus ([Table 3](#)). The
309 elastic modulus of longitudinal PM was significantly different between fresh and flash frozen samples
310 (17.2 ± 9 MPa against 21.3 ± 9.1 MPa respectively) ([Table 3](#)).

311 The CSA were used to choose the most likely CSA of tested samples from the probabilistic
312 distribution computed from a Monte-Carlo approach of uncertainties quantification. Then the

313 probabilistic range of elastic moduli computed from the 38th (E_38) and 62th (E_62) percentile
314 probabilistic values of CSA is depicted in the [Table 3](#). The range of probabilistic values of elastic
315 modulus differ from the standard deviation range. Indeed, the probabilistic elastic moduli range (E_32
316 – E_68) was greater than the range of SD experimental elastic moduli (E) for DAC samples in
317 circumferential direction. In longitudinal direction, the inferior value of probabilistic range (E_62) was
318 greater than the SD inferior value of DAC samples. For the PM samples in longitudinal direction, E_38
319 and E_62 values were respectively always greater to the superior and the inferior SD values.

320 Ogden coefficient presented in Table. 5 were computed for curves (min, max and typical) of the
321 Fig. 4 and were represented in Fig. B (Appendix B).

322 Typical stress and strain fields were depicted in [Appendix C as well as the maximum deformations](#)
323 [to failure in Table B.1 \[Appendix B\]](#). The maximum stresses and strains are not located in the same
324 part of the sample describing the heterogeneous behavior of the DAC and PM tissues.

325 **4. Discussion**

326 The elastic mechanical properties of meninges along the porcine spinal cord were quantified and
327 tested in the longitudinal and the circumferential directions. While assuming a linear elastic behavior only
328 provides a macroscopic mechanical description of only the linear part of the stress/stress curve. The results
329 showed that the cervical DAC linear modulus is the lowest in longitudinal loading direction while the
330 lumbar DAC linear modulus is the highest in circumferential loading direction. The effect of conservation
331 method between fresh and flash frozen (at-80°C) samples at the thoracic level showed suggest the
332 reliability of the flash frozen conservative approach for DAC tissues. To the best of our knowledge, such
333 comparison of mechanical properties between the cervical, thoracic and lumbar spinal levels as well as of
334 the behavior of flash frozen with fresh samples is an addition to the current literature. A probabilistic
335 quantification of CSA was performed to estimate the effect of measurement variabilities reported for the
336 thickness and the width, resulting in a probabilistic elastic moduli per tissue and loading direction.

337 For the next paragraphs, the literature comparisons were solely focused on spinal meninges
338 mechanical properties, without any comparison with the brain meninges properties. This choice was done
339 due to the difference of structural tissue alignments.

340 **4.1. Spinal levels**

341 *4.1.1. Dura-Arachnoid Complex (DAC)*

342 The significant mechanical difference between the porcine DAC elastic moduli tested in
343 longitudinal and circumferential loading directions matched the mechanical spinal dura mater
344 behavior previously reported in literature. However, the magnitude of our elastic moduli was lower.
345 Our mean longitudinal elastic modulus of DAC was 1.8 times significantly superior to the
346 circumferential elastic modulus at the thoracic spinal level while the longitudinal elastic modulus was
347 three times significantly superior to the circumferential elastic modulus at the lumbar spinal level
348 which is lower than literature measurements. The longitudinal over circumferential elastic moduli
349 ratio is still coherent (Patin et al. 1996, Zarzur 1996, Runza et al. 1999, Yang et al. 2019). Then, our
350 results in terms of elastic modulus support a longitudinal fiber alignment for the pigs, in accordance
351 with the literature for the rat (Maikos et al. 2008), the dog (Patin et al. 1996), for the pig (Kinaci et al.
352 2020) and for the human (Chauvet et al. 2010). We showed elasticity variations along the canal for the
353 DAC in longitudinal and in circumferential loading directions. The greatest elasticity in longitudinal
354 loading direction was measured at the cervical spinal level whereas the greatest elasticity in the
355 circumferential loading direction was measured at the lumbar level.

356 In longitudinal loading direction for DAC samples, significant differences were highlighted in our
357 study between each spinal level of elastic moduli. The reported longitudinal elastic modulus at the
358 lumbar level of human cadavers varied between 65 and 102 MPa for fresh samples (Runza et al. 1999)
359 whereas it was 36.6±12.6 MPa in our study in similar testing conditions. A similar difference is
360 observed between the frozen samples of human cadavers varying between 42 and 142 MPa (Runza et
361 al. 1999) whereas it was 39.3±19.3 MPa for flash frozen samples in our study. In circumferential
362 loading direction, the DAC elastic modulus was found significantly lower at the lumbar level in this

363 study when compared to cervical and thoracic levels. These differences of elasticity could be due to
364 local structural changes at the cervical and lumbar levels in order to respectively support the
365 longitudinal and the circumferential loading. Indeed, the particular local geometry around the
366 foramen magnum and around the cauda equina would induce these local structural changes, locally
367 modifying the mechanical properties. A loss of a longitudinal alignment at the cervical and the lumbar
368 spinal levels could explain a loss of cervical longitudinal and lumbar circumferential elasticity.

369 Firstly, a potential link between the tissue stiffness variation and the range of motion of each
370 spinal segment could explain such findings. Indeed, the absence of significant difference between the
371 longitudinal and the circumferential loading orientation at the cervical level of DAC could be explained
372 by the variation of range of motion (ROM) between the spinal segments (cervical, thoracic, lumbar).
373 Indeed, the ROM in flexion/extension, lateral bending angles are greater at the cervical level (Wilke et
374 al. 2011, Persson et al. 2010). The capacity to flex and to bend of the neck do not induce any
375 significant difference of elastic mechanical properties in longitudinal and in circumferential directions.
376 Then, the lower ROM in the thoracic and lumbar spinal levels bringing a greater stability (Wilke et al.
377 2011) limits the circumferential the stresses and the strains in the canal. For the dura mater, this could
378 result in a higher capacity to be stressed in the longitudinal orientation.

379 Secondly, the higher values of circumferential stiffness of the DAC were found at the cervical and
380 thoracic levels. The high or reasonable presence of cerebrospinal fluid (CSF) vortices and the higher
381 CSF pulsatile pressure in the canal of the cervical and thoracic segments compared to the lumbar
382 segment (Tangen et al. 2015, Khani et al. 2018) could explain such observations. Indeed, these
383 potential stresses applied by the fluid vortices (orthogonally to the longitudinal flow direction) on the
384 DAC in the upper spinal levels could create a tissue densification driving to a greater circumferential
385 rigidity. This assumption takes its source from the Davis 'law (the corollary of Wolff's law for soft
386 tissue) (Davis 1867) and from others mechanobiological theories (Liedekerke et al. 2019, Cyron and
387 Humphrey 2017).

388 4.1.2. *Pia Mater (PM)*

389 In PM samples, no significant difference was observed between the elastic moduli of each spinal
390 level. There is a limited number of studies on the PM tissue material properties compared to the dura
391 mater. Accordingly, no comparative data was found. However, two studies investigating pia mater
392 properties performed on ewe and rabbit model were used as partial comparative data. Our results
393 show a significantly higher value of elastic modulus (18.4 ± 8 MPa) compared to the elastic modulus of
394 cervical rabbit (2.4 MPa) tested with a ramp of 0.02 N/s (Ozawa et al. 2011). This difference can be
395 explain by the inter-specie ROM, the motion strategy and the morphology between the rabbit and the
396 porcine model. However, our values are coherent with the modulus (17 MPa) of the cervical ewe PM
397 samples tested at strain rate of 0.05/s (Ramo et al. 2018c). Ozawa et al. (2011) showed that the rabbit
398 pia mater increased the stiffness of the spinal cord and enhanced its shape recovery after releasing the
399 compression on the spinal cord. The porcine PM sheath seems also maintain the oval spinal cord
400 shape with a similar elasticity. Then the Poisson's effect of the human spinal cord (Breig and Braxton

401 1960, Sudres et al. 2019) could be limited by the PM mechanical properties. The PM would mitigate
402 the stresses and strains into the spinal cord during posture changes.

403 **4.2. Protocol aspects**

404 4.2.1. Testing model

405 Due to morphological, feasibility, housing, ethical in term of accessibility to human cadavers,
406 histological (Kinaci et al. 2020) and biomechanical considerations in term of the cervical and thoracic
407 ROM in lateral bending and in flexion/extension (Wilke et al. 2011), this quadruped makes the most
408 appropriate transversal models human, next to non-human primates (Schomberg et al. 2017).

409 4.2.2. Failure pattern aspects

410 One of the limitations of our protocol is the method used to cut our samples. That could generate high
411 damaging risks, making it difficult to homogenize the dimensions of each samples. The consequence of
412 that is with the occurrence of failure at the level of clamps in a majority of samples, induced by stress
413 concentration by the jaws [Fig. C.5]. When failure occurred in the center of samples [Fig C.6 – B, C], we
414 observed a difference in failure pattern between the DAC in circumferential loading and the PM in
415 longitudinal loading. Indeed, PM appeared to be more heterogeneous with vessels more apparent
416 while the DAC appeared more homogeneous macroscopically with an assumed longitudinal fiber
417 organization.”.

418 Additional stress and strain fields analyses were proposed on typical samples (Fig C.1-3). Two
419 solutions could solve this protocol issue: cutting using the sample with a steel mechanical cutting piece
420 (Chauvet et al. 2010) and the fastening of the sample to the clamps with a pressure sensor, thus
421 ensuring symmetrical boundary conditions. In addition, working in an environmental chamber with
422 CSF-like fluid could maintain moisture conditions of the sample and reproduce a more biofidelic test
423 environment. However, those solutions would certainly induce longer protocols, worsening the *post*
424 *mortem* degradation of tissues.

425 4.2.3. Conservative approaches

426 Regarding conservative method aspect, our value of elastic moduli as well as our values of
427 damage stresses are lower than the mechanical properties provided in the literature (Runza et al.
428 1999). This difference could be due to the time and the choice of conservation (mean time after
429 euthanasia: mean of 7h26 against between less than 24h and 120h of low temperature freezing). The
430 classical freezing at -4°C seems to result in a drying of the sample and to finally increase the stiffness
431 of the samples. Mazgajczyk et al. (2012) showed cervical stress and strain values greater than our
432 measurements. The difference of damage or maximum stresses could be explained by the porcine
433 species used (Yorkshire X Landrace against no detailed porcine animals), the maximum time used to
434 perform the experiments (12h23 against 24h) and the conservation method (flash freezing at -80°C
435 against a classical freezing method below 0°C without detail).

436 No significant difference was mechanically observed between the fresh and flash-frozen DAC
437 samples' elastic moduli. This could be explained by a denser fiber of the DAC structure compared to
438 the PM structure (Reina et al. 2020). This higher density of fibers could limit the effect of freezing at

439 very low temperature, in limiting the shrinkage of the matter caused by H₂O presence and in limiting
440 the quantity of fluid available in the tissue. The flash-freezing conservative approach allowed to
441 reduce the importance of the time variable in an experimental protocol, as well as to mitigate the
442 mechanical behavior between the flash frozen and the fresh samples. In addition, both tissues have
443 viscoelastic properties due to their direct contact with the cerebrospinal fluid. A further study
444 investigating the viscoelastic properties differences between fresh and flash frozen samples could
445 highlight the effect of fluids with respect to the tissue structure in the mechanical behavior of spinal
446 meninges. Then, the flash freezing increased the stiffness of PM samples with respect to the fresh
447 samples. A further study could investigate this effect potentially induced by a limited number of flash-
448 frozen PM thoracic samples.

449 4.2.4. *Thickness uncertainty analysis*

450 The measurements of force/displacement of the samples remain a challenge, particularly due to
451 uncertainty errors induced by the protocol. The thickness measurements were not performed on the
452 tested samples but on one specific porcine subject not included in the mechanical testing protocol due
453 to the high damaging risk induces by tissue manipulation. Measurement errors from the tools as well
454 as the difference of sample thickness measurement (with a different pig to avoid damaging the
455 sample) could lead to non-linear result uncertainties ([Appendix A – Fig. A.2](#)). A propagation
456 uncertainty analysis is not frequently used for soft tissue characterization, but it brings a more
457 complete and comparable range of mechanical properties. The GUM uncertainty analysis ([ISO 98:1995](#)
458 [GUM](#)) provided the uncertainty distribution ([Appendix A – Fig. A.1](#)) of CSA as an output and from input
459 uncertainty distributions of thickness and width repeatability and width resolution. Such output CSA
460 uncertainty distribution is then used to the probabilistic elastic moduli calculations. The more the
461 input uncertainty is clearly defined, the more the output uncertainty distribution will be accurate. As
462 depicted in Table 3, differences between the probabilistic moduli (E₃₂ and E₆₈) and the
463 experimental elastic modulus (E) varied with respect to the type of tissue (DAC and PM) and with
464 respect to the loading direction (longitudinal or circumferential). When focusing on the existing
465 available elastic moduli values in the literature, no uncertainties are provided and the provided SD
466 ranges of experimental elastic moduli should then be taken carefully.

467 To experimentally face the thickness measurement challenge, the implementation of a non-
468 contact measurement method (laser) during the tensile test could be investigated. Otherwise, a
469 combined approach between geometrical and biomechanical experiments would allow to establish
470 potential correlation between geometrical and biomechanical behavior of the meninges as proposed
471 by [Yoganandan et al. \(2000\)](#) for the cervical human ligaments.

472 4.2.5. *Strain rate and linear elasticity approximation*

473 Only one moderate strain rate (0.2 mm/s) was used to describe the mechanical
474 properties of the spinal meninges along the spinal cord which would correspond to a
475 value lower than those occurring during injury [[Ramo et al. 2018b](#)].

476 Previously reported uniaxial tensile tests showed no significant strain rate
477 dependency at low strain rate and moderate strain rates (0.01s⁻¹, 0.1s⁻¹ and 1s⁻¹) for
478 dura mater [Persson et al.2010]. The same finding was reported for the pia-arachnoid
479 complex at 0.016 and 0.16 s⁻¹ [Ramo et al. 2018c]. However, strain dependency of the
480 meninges should be investigated more in details in future work. In addition, the elastic
481 properties are easily comparable with the existing literature [Zwirner et al. 2019, Runza et
482 al. 1999, Ramo et al. 2018c, Ozawa et al. 2011]. A potential correlation between “the
483 higher nonlinearity observed in the circumferential direction as compared to longitudinal
484 direction” due to the deformation of the toe region and the low deformations of the
485 spinal cord in physiological conditions. gradual to region due to the toe region during
486 physiological motion of the spinal cord was discussed in literature [Shetye et al. 2014]. We
487 assumed the linear part would be a transitional phase (between the physiological
488 behavior of the tissue and a beginning failure phase) in which the tissue is not yet injured.
489 We additionally provided a range of isotropic hyperelastic (one term Ogden model)
490 coefficients to provide a better description of the tissue behavior in all test loadings
491 direction for the first 15% of strain.

492 The μ Ogden coefficients of the typical curves were found superior to those provided
493 for spinal bovine dura mater at 0.01 s⁻¹ (5.0 MPa against almost null in the longitudinal
494 direction and 2.4 MPa against 2.2 MPa in the circumferential direction respectively) . The
495 b Ogden coefficients were found inferior to those provided in the same study (11.9
496 against almost 24.0 in the longitudinal direction and 10.5 against 18.0 in the
497 circumferential direction respectively) [Persson et al. 2010]. No equivalent data was found
498 to compare the Ogden coefficients of PM.

499 Trends reported in inter-segmental spinal level variation (cervical, thoracic and
500 lumbar) of elastic properties in DAC and PM need to be confirmed due to high standard
501 error induced by within-subject and between-subject variability. Thus a larger population
502 of samples by levels and by subject will be tested. Anisotropic hyperelastic models [De
503 Kegel et al. 2018, Shetye et al. 2014] as well as dorsal and ventral locations of samples
504 along the spine [Mazgajczyk et al. 2012] could be investigated in future work. Finally, our
505 mechanical properties could be used in spine FE models such as stenotic scenarios [Bailly
506 et al. 2020] or post-surgery analysis [Stoner et al. 2020] for a better description of tissues
507 in longitudinal and circumferential loading direction of DAC and in longitudinal PM.

508 **5. Conclusion**

509 The porcine model is an appropriate alternative to study the mechanical properties of meninges
510 compared to human cadavers. In addition, this study provided isotropic hyperelastic properties and
511 showed the negligible effect of a flash freezing conservative method to provide comparable results on
512 DAC tissues, thus limiting the effect of the time factor in such experimental protocol.

513 The following mean values were proposed for the elastic moduli of the spinal meninges:

- 514 • Longitudinal DAC elastic moduli: **22.4 MPa** (SE: 23.0) in cervical, **38.1 MPa** (SE: 23.3) in
515 thoracic and **36.6 MPa** (SE: 23.81) in lumbar spinal levels.
- 516 • Circumferential DAC elastic moduli: **20.6 MPa** (SE: 6.6) in cervical, **21.2 MPa** (SE: 6.7) in
517 thoracic and **12.2 MPa** (SE: 6.6) in lumbar spinal levels.
- 518 • Longitudinal PM elastic moduli: **18.4 MPa** (SE: 14.4) in cervical, **17.2 MPa** (SE: 13.7) in
519 thoracic and **19.6 MPa** (SE: 13.5) in lumbar spinal levels.

520 This variety of mechanical properties of the meninges suggests that it cannot be regarded as a
521 homogenous structure along the whole length of the spinal cord. Furthermore, a spatial
522 personalization along the spinal cord of this biological soft elastic behavior should be taken into
523 account when building a numerical model of the central nervous system.

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Level	Tissue	Fresh samples			Flash Frozen samples		
		Nb of tested samples	Nb of excluded	Nb of analyzed	Nb of tested samples	Nb of excluded	Nb of analyzed
Cervical	LONG DAC	15	1	14		X	
	CIRC DAC	20	3	17		X	
	LONG PM	14	0	14		X	
Thoracic	LONG DAC	15	3	12	9	3	6
	CIRC DAC	8	0	8	11	0	11
	LONG PM	18	1	17	13	5	8
Lumbar	LONG DAC	10	0	10		X	
	CIRC DAC	12	1	11		X	
	LONG PM	16	0	16		X	
Total	LONG DAC	40	4	36	9	3	6
	CIRC DAC	40	4	36	11	0	11
	LONG PM	48	1	47	13	5	8
Total		128	9	119	33	8	25

Table 1. Summarized description of number of samples

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Level	Tissue	Length [mm]	Width [mm]	Thickness [mm]	Cross-sectional area [mm ²]
Cervical	LONG DAC	9.65 (0.96) [8.33-12.06]	11.35 (2.22) [8.1-15.73]	0.28 (0.05) [0.22-0.37]	3.18 (0.62) [2.27-4.40]
	CIRC DAC	10.61 (1.95) [8.19-15.92]	11.16 (2.75) [7.2-15.02]	0.28 (0.05) [0.22-0.37]	3.12 (0.77) [2.02-4.21]
	LONG PM	9.1 (1.7) [6.99-12.7]	8.09 (1.93) [3.96-10.92]	0.22 (0.04) [0.16-0.28]	1.78 (0.42) [0.87-2.40]
Thoracic	LONG DAC	11.7 (1.81) [9.23-15.36]	11.79 (4.6) [7.66-22.44]	0.32 (0.05) [0.19-0.49]	3.77 (1.47) [2.45-7.18]
	CIRC DAC	11.18 (2.22) [9.14-15.6]	15.12 (3.62) [9.34-20.98]	0.32 (0.05) [0.19-0.49]	4.84 (1.16) [2.99-6.71]
	LONG PM	10.65 (1.61) [8.1-13.75]	12.13 (3.75) [4.5-16.21]	0.2 (0.05) [0.11-0.29]	2.44 (0.76) [0.90-3.24]
Lumbar	LONG DAC	12.35 (1.92) [10.08-15.49]	9.64 (1.69) [7.94-12.57]	0.28 (0.05) [0.21-0.37]	2.7 (0.47) [2.22-3.52]
	CIRC DAC	11.36 (3.44) [6.36-18.51]	12.03 (3.01) [6.88-6.69]	0.28 (0.05) [0.21-0.37]	3.37 (0.84) [1.93-4.67]
	LONG PM	11.13 (2.14) [6.23-13.91]	8.88 (2.35) [4.79-12.55]	0.2 (0.04) [0.14-0.28]	1.78 (0.47) [0.96-2.51]

Table 2. Summary of sample dimensions. Each quantity is described as the mean value, the standard deviation between parenthesis and the min and max respectively, between brackets.

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Level	Tissue	Toe strain (ϵ_{toe})	Toe stress (σ_{toe}) [MPa]	Failure strain ($\epsilon_{failure}$)	Failure stress ($\sigma_{failure}$) [MPa]	Elastic modulus (E) [MPa]	E_0.32 [MPa]	E_0.68 [MPa]
Cervical	LONG DAC (n=14)	0.04 (0.03)*T	0.5 (0.21)	0.25 (0.09)*T,L	5.08 (2.97)	22.3 (10.0)*T,L (SE: 23.0)	30.3	17.4
	CIRC DAC (n=17)	0.04 (0.02)	0.49 (0.37)	0.23 (0.1)*L	4.1 (2.23)	20.6 (8.1)*L (SE: 6.6)	49.5	25.8

	LONG PM (n=14)	0.03 (0.01)*T,L	0.64 (0.27)*T,L	0.23 (0.08)	3.85 (1.88)	18.4 (8.0) (SE: 14.4)	30.4	16.0
	LONG DAC (n=12)	0.02 (0.01)*C	0.49 (0.33)	0.15 (0.09)*C	4.98 (2.7)	38.1 (12.6)*C,§ (SE: 23.3)	69.1	27.4
Thoracic	CIRC DAC (n=8)	0.03 (0.01)	0.27 (0.07)	0.21 (0.08)*L	3.94 (1.38)*L	21.2 (3.0)*L,§ (SE: 6.7)	30.4	16.6
	LONG PM (n=17)	0.02 (0.02)*C	0.34 (0.18)*C	0.2 (0.05)	3.07 (1.73)	17.2 (9) (SE: 13.7)	28.4	11.6
	LONG DAC (n=10)	0.03 (0.01)	0.73 (0.41)	0.18 (0.09)*C	5.3 (1.62)	36.6 (12.6)*C,§ (SE: 23.8)	45.2	26.8
Lumbar	CIRC DAC (n=11)	0.03 (0.01)	0.3 (0.11)	0.32 (0.14)*C,T	3.26 (0.89)*T	12.2 (4.4)*C,T,§ (SE: 6.6)	17.2	8.9
	LONG PM (n=16)	0.02 (0.01)*C	0.43 (0.2)*C	0.18 (0.03)	3.17 (0.91)	19.6 (6.8) (SE: 13.5)	31.1	15.2
	LONG DAC (n=36)	0.03 (0,02)	0.56 (0.32)	0.2 (0.1)	5.11 (2.5)	32.3 (13.5) (SE: 21.7)		
Total	CIRC DAC (n=36)	0.03 (0,02)	0.38 (0.28)	0.25 (0.12)	3.81 (1.74)	18.0 (7.3) (SE: 6.53)		
	LONG PM (n=47)	0.02 (0,01)	0.46 (0.25)	0.2 (0.06)	3.34 (1.56)	18.4 (7.9) (SE: 10.2)		

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Mean with standard deviation in parenthesis values as well as reported standard error (SE).

Bold*C,T,L stand for a significant difference between the spinal level and the level given by the letters C (cervical),T (thoracic) or L (lumbar) for a given tissue and a given variable. ($p<0.05$)

Bold § stands for a significant difference between the DAC longitudinal and circumferential for a given spinal level ($p<0.05$).

Table 3. Summary of post-processed quantities by spinal segment (stress, strain, elastic moduli and probabilistic elastic moduli) for toe region, quasi-linear region and damage region.

Level	Tissue	Elastic Modulus [MPa]	
		Fresh samples	Flash Frozen samples
Thoracic	LONG DAC	38.1 (12.6) n=12	39.3 (19.3) n=6
	CIRC DAC	21.2 (3) n=8	17.4 (4.4) n=11
	LONG PM	17.2 (9) n=17	21.3 (9.1) n=5

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Bold* stands for a significant difference between the fresh and the flash-freezing samples for the thoracic level and a given tissue ($p<0.05$).

Table 4. Comparison table of samples's elastic modulus between fresh and flash-freezing conservation methods.

Authors	Journal	Number of specimen ; samples	Specie	Tissue	Spinal level	Type of test	Strain rates	Conservation method	Elastic modulus	
									Longitudinal	Transverse
Patin <i>et al.</i> 1993	Anesthesia & Analgesia	9 specimens ; 7 samples by specie	human dog	dura mater	lumbar	uniaxial tension	1.67 mm/s	Refrigerated in saline solution	human : 138 - 265 MPa dog : 58.8 - 73.5 MPa	human : 7.8 - 76.4 MPa dog : 54.9 - 58.8 MPa
Runza <i>et al.</i> 1999	Anesthesia & Analgesia	6 cadavers ;	human bovin	dura mater	T12 - L4/L5	uniaxial tension	10 mm/min	In saline solution , frozen at 4°C for 24h and 120h	human : 65 - 102 MPa bovin : 25 - 80 MPa	human : 5 MPa
Maikos <i>et al.</i> 2008	Journal of Neurotrauma	23 spinal samples	rat	dura mater	C1 - L1	uniaxial tension	19.4 sec-1; 0.0014 sec-1	2h after sacrifice		
Mazgajczyk <i>et al.</i> 2012	Acta of bioengineering and biomechanic	9 specimens ; 250 samples	porcine	dura mater	C1 - C7	uniaxial tension	2 mm/min	Frozen		
Chauvet <i>et al.</i> 2010	Neurosurgical Review	10 specimens; 22 samples	human	dura mater	Craniocervical junction	uniaxial tension		Fresh cadaver	entire dura : 44 - 91 MPa split dura : 19 - 25 MPa	
Yang <i>et al.</i> 2019	Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi	5 specimens	sheep	dura mater	C6, C7, T11, T12, L4, and L5	uniaxial tension	0.016 mm/s	in saline solution	cervical : 15.97±2.52 MPa thoracic : 12.39±1.88 MPa lumbar : 8.33 ±3.10 MPa	cervical : 14.38±2.68 MPa thoracic : 8.78±1.01 MPa lumbar : 3.46±1.24 MPa
Ozawa <i>et al.</i> 2011	Journal of Neurosurgery	9 specimens	rabbit	pia mater	C5 - C6	uniaxial tension and compression	0.02 N/s	In saline solution	2.4 MPa	
Ramo <i>et al.</i> 2018c	Acta Biomaterialia	8 specimens	ewe	pia-arachnoid complex	C0 - C7	uniaxial tension	0.05sec-1	fresh after sacrifice	17 MPa	

Table 5. Comparative literature table of mechanical characterization of spinal meninges

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Loading direction/ Tissue	μ	b	R^2	RMSE
Longitudinal DAC				
Curve 1 (superior limit of the corridor)	6.619 (6.452, 6.787)	11.8 (11.36, 12.24)	0.9974	0.0739
Curve 2 (typical curve)	5.02 (4.882, 5.158)	11.93 (11.45, 12.41)	0.9966	0.0613
Curve 3 (inferior limit of the corridor)	3.286 (2.966, 3.606)	-7.291 (-16.47, 1.888)	0.9916	0.0292
Circumferential DAC				
Curve 1 (superior limit of the corridor)	4.684 (4.562, 4.806)	6.955 (6.379, 7.531)	0.9973	0.0396
Curve 2 (typical curve)	2.499 (2.397, 2.6)	10.5 (9.761, 11.24)	0.9942	0.0377
Curve 3 (inferior limit of the corridor)	0.765 (0.74, 0.79)	10.77 (10.18, 11.36)	0.9946	0.0109
Longitudinal PM				
Curve 1 (superior limit of the corridor)	14.12 (13.88, 14.36)	7.342 (6.972, 7.712)	0.9984	0.0885
Curve 2 (typical curve)	4.184 (4.04, 4.329)	9.01 (8.338, 9.683)	0.9966	0.0437
Curve 3 (inferior limit of the corridor)	3.763 (3.684, 3.842)	2.549 (1.927, 3.172)	0.9977	0.0235

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564 **Table 6. One degree Ogden model material constants b and μ the shear modulus of the curves depicted in Fig. 4 and Fig. B (Appendix B).**
565 **A 95% confidence interval is provided between parenthesis for each couple of the material constants.**

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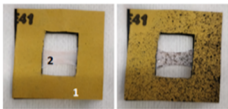
Figures

Figure 1. Mechanical testing set up: A/ (1) Sandpaper support with (2) fastened sample (left), with stochastic pattern (right) ; B/ Ramp test to-failure. (3) Clamps, (4) load cell ; C/ Lateral view of the setup with (5) a spotlight, (6) a white filter for indirect lightening of the sample ; D/ Front view including the (7) mechanical testing machine, (8) a high-definition camera, (9) a sliding support to adjust the location of the camera and (10) a computer to control and to record the measurement.

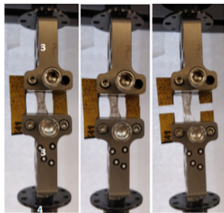
Figure 2. Typical imposed displacement over time for each mechanical tensile test. 1) Initial pre-load, 2) 30 preconditioning cycles, 3) ramp to failure.

Figure 3. Identification of the linear elastic region of interest. A- force/displacement responses with two elastic limits delimiting the three regions (1 - toe region ending by the toe force and the toe displacement, 2 – linear elastic region, 3 – failure region starting by the failure force and the failure displacement) and the linear regression, B) 2nd derivative of the force/displacement curve identifying the elastic limits points.

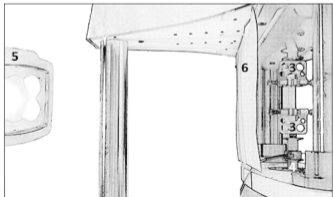
Figure 4. Mechanical of the tissue samples (shaded and curves): A - longitudinal DAC ; B - circumferential DAC ; C - longitudinal PM). Dotted lines represent the maximum and the minimum stress/strain curves delineating the experimental corridor (shaded area) and the continuous lines represent the typical stress/strain curves for A, B and C.



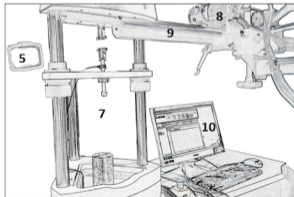
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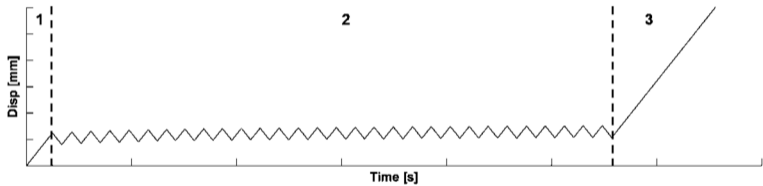
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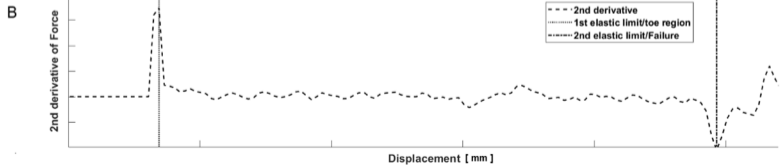
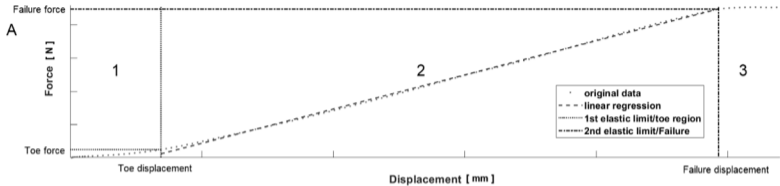


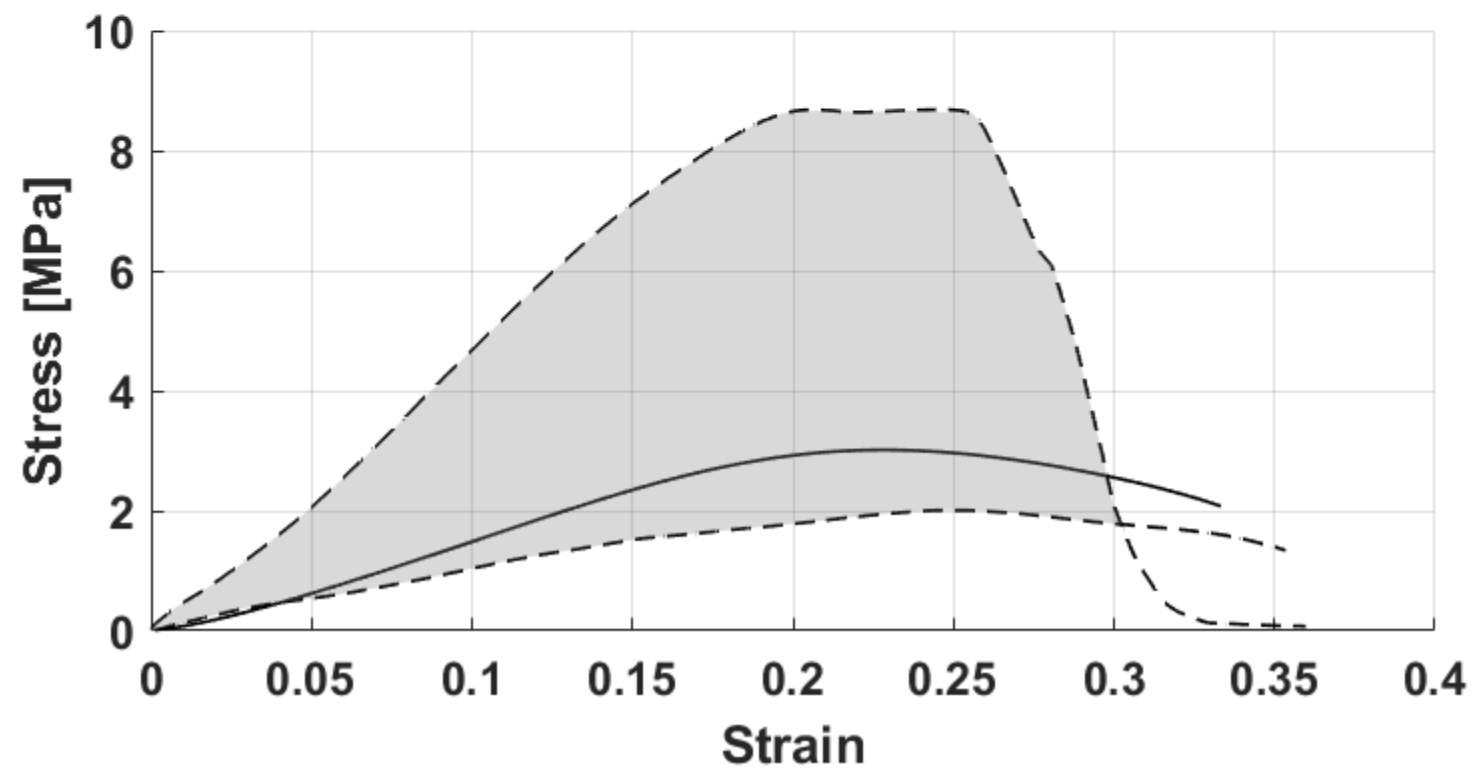
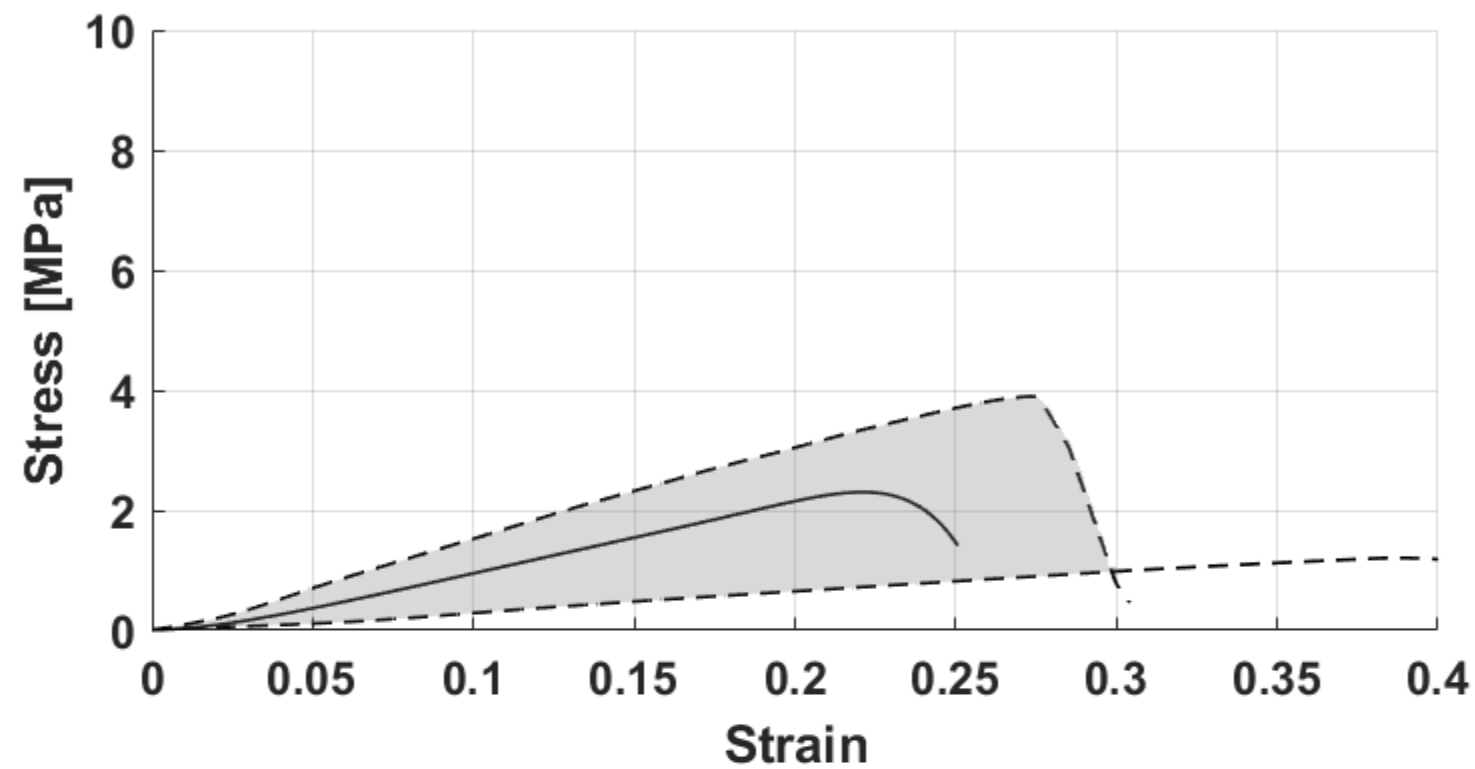
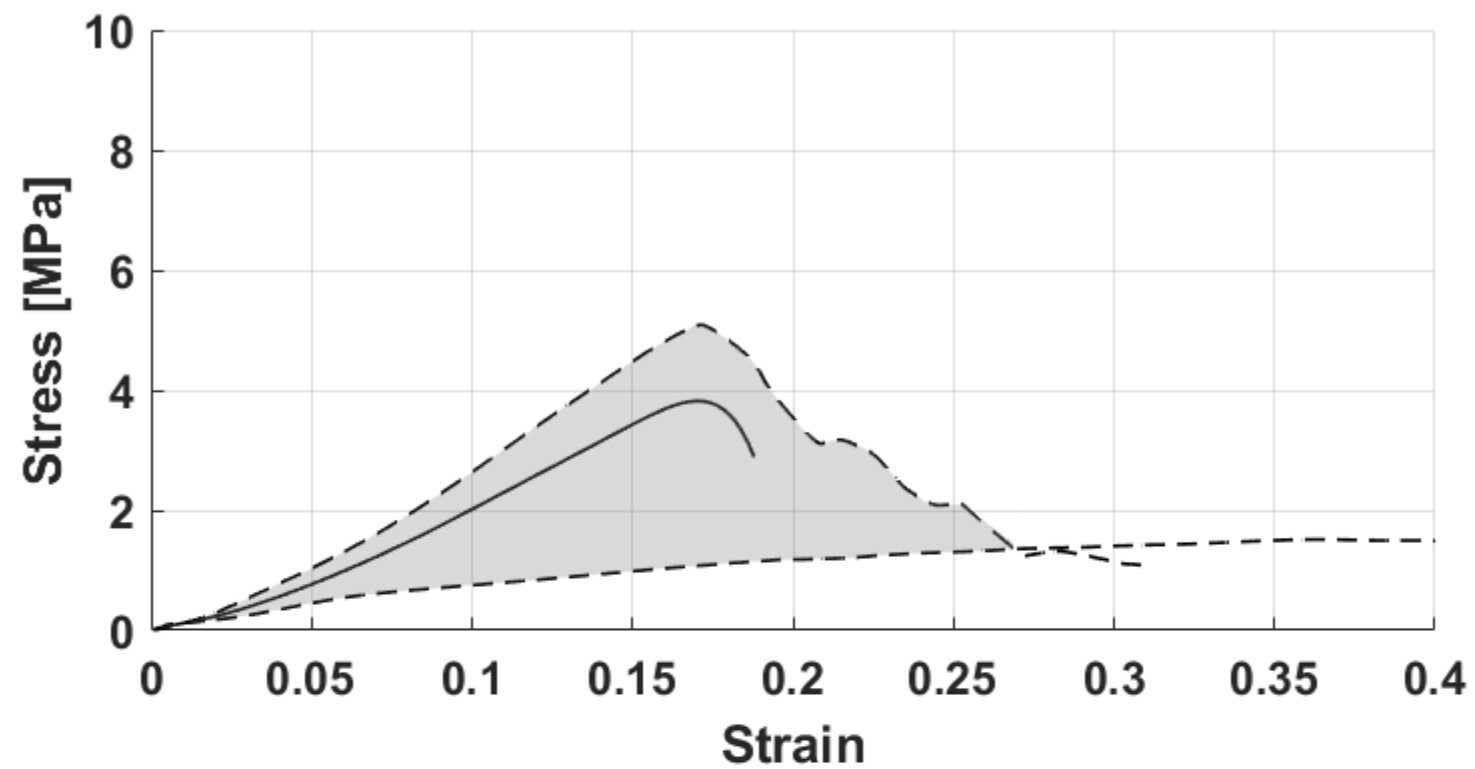
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D







— Typical strain/stress curve
- - - Minimum and Maximum strain/stress curves

Dissection and preparation



Tensile mechanical test

