Acta of Bioengineering and Biomechanics
Vol. 15, No. 1, 2013

Original paper
DOI: 10.5277/abb130107

Spinal sections and regional variations in the mechanical properties of the annulus fibrosus subjected to tensile loading

MAŁGORZATA ŻAK, CELINA PEZOWICZ*

Division of Biomedical Engineering and Experimental Mechanics, Department of Mechanical Engineering, Wrocław University of Technology, Poland.

The annulus fibrosus is the primary load-bearing component of the intervertebral disc responsible for proper transfer of loads in the spine. The aim of this study was to determine selected mechanical parameters of multilayer specimens of the annulus fibrosus of the intervertebral disc during uniaxial tensile loading. The anatomical location (anterior/posterior) of the test specimens of the annulus fibrosus and its location along the length of the spine were analysed to determine their impact on the maximum failure force, stiffness, the value of Young's modulus and dissipated energy. The results indicated high energy losses over the five consecutive precondition loops while the value of the force remained at a constant level. The thoracic and lumbar specimens showed the highest values of the parameters analysed. There were also significant changes depending on the anatomical region of the intervertebral disc, where anterior specimens demonstrated higher mechanical values compared to posterior specimens.

Key words: *intervertebral disc, annulus fibrosus, tensile properties, dissipated energy, hydration*

1. Introduction

The annulus fibrosus is the primary component of the intervertebral disc. In addition to collagen fibres, it contains elastin fibres, water, proteoglycans and non-collagenous proteins. However, it is collagen fibres, forming a matrix of consecutive, concentrically arranged annulus fibrosus lamellae, that determine the proper functioning of the entire intervertebral disc.

The high strength of the intervertebral disc is related to maintenance of a balance between the intradiscal pressure, caused by a complex system of forces acting on the spine, and direct anchoring of collagen fibres of the outer part of the annulus fibrosus (2/3) in the bone tissue of vertebral bodies adjacent to the disc. This provides additional, significant enhancement of the disc-vertebral body connection under the impact of tensile and shearing forces acting on motor segments [19], [27], [34].

The mechanical properties of the annulus fibrosus depend on its position in a given segment of the spine due to, among others, different values of the forces loading a particular segment [28] as well as its connection within the area of the intervertebral disc itself (anterior or posterior part) [21], [33].

Variability of strength and structural properties of the annulus fibrosus within the area of the disc has been demonstrated in a number of research works [1], [3], [11]–[16], [18], [30]. Most of them represent analyses of the mechanical properties conducted in uniaxial tensile tests. The tests usually involve multilayer fragments of the annulus stretched in the circumferential or vertical direction, and less frequently single annulus lamellae [16], [30]. The test specimens are excised from isolated intervertebral discs. However,

* Corresponding author: Celina Pezowicz, Division of Biomedical Engineering and Experimental Mechanics, Wrocław University of Technology, ul. Łukasiewicza 7/9, 50-371 Wrocław, Poland. Tel:+48 71 320 27 13, e-mail: celina.pezowicz@pwr.wroc.pl
Received: July 16th, 2012
Accepted for publication: November 17th, 2012

the specimens rarely have preserved attachments in the bone part of vertebral bodies [6], [16], [24]. This somewhat limits the possibility of identifying changes at the vertebral body- disc-vertebral body complex, which is regarded as the primary functional unit of the spine [36]. Parameters obtained in strength tests of the annulus fibrosus include, e.g., maximum tensile force, elastic modulus or changes in strain and stress. However, taking into account viscoelastic properties of the annulus fibrosus, it is important to understand the nature of changes taking place in this structure during the so-called preconditioning, i.e., loads prior to the final strength test [37].

The main aim of this study was to characterize the mechanical properties of multilayer specimens of the annulus fibrosus with regard to differentiation of the anatomical regions and the spinal segments. Another major aim of this study was an analysis of the parameters determining tensile properties in the form of changes in dissipated energy determined in initial cycles (preconditioning).

2. Materials and methods

The research material consisted of spines obtained *post-mortem* from 3 domestic pigs aged 8 months, with an average weight of about 130 kg, coming from a single breeder. Specimens of animal origin are commonly used as research models, successfully replacing human (cadaver) specimens [4], [8], [31], [32].

After collection, the specimens were cleaned off soft tissue and posterior bone elements (arches with processes) were removed so as to leave exposed the structure of the vertebral bodies and the intervertebral disc. Then, the specimens were divided into 3 segments: cervical, thoracic and lumbar.

Single motor segments were isolated in each of the segments, consisting of an intervertebral disc together with adjacent halves of vertebral bodies (body-disc-body). The study was conducted for 3 selected segments coming from consecutive segments of the spine, i.e.: cervical C3-C4, C4-C5 and C5-C6; thoracic Th8-Th9, Th9-Th10 and Th10-Th11; and lumbar L2-L3, L3-L4 and L4-L5. The material prepared was frozen in separate plastic packaging at a temperature of –20 °C until testing.

Specimens were excised from each segment, which contained the outer part of the annulus fibrosus. Lamellae of this part of the annulus fibrosus are homogeneous in terms of arrangement and density of collagen fibres compared to the annuli situated internally, which are loosely and heterogeneously arranged collagen fibres [21], [25], [33]. Anterior (A) and posterior (P) margins were excised from frozen specimens in the frontal plane, which were then divided into two symmetrical parts, Fig. 1.

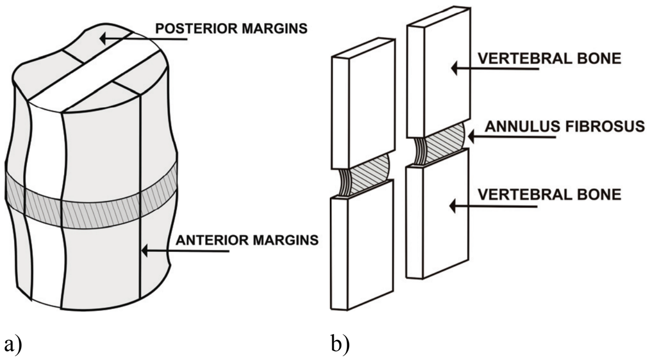

Fig. 1. Diagram of sampling from the spinal segment: (a) anterior and posterior margins; (b) single specimens of outer layers of the annulus fibrosus together with bone insertion

Individual specimens were prepared in such a way as to obtain similar geometrical dimensions of the annulus, in particular thickness, which would correspond to a comparable number of layers of the annulus fibrosus lamellae in each of the specimens. As a result, 54 multilayer annulus fibrosus specimens of a non-degenerated intervertebral disc were obtained, 18 specimens per each segment with the average dimensions:

Table 1. A comparison of average dimensions of the specimens depending on the sampling site and segment. Values are the mean ±SD for all 3 spinal segments

Spinal segments	Cervical		Thoracic		Lumbar	
Location of the annulus fibrosus	anterior ($n = 9$)	posterior ($n = 9$)	anterior ($n = 9$)	posterior ($n = 9$)	anterior ($n = 9$)	posterior ($n = 9$)
Length [mm]	6.33 ± 0.98	5.79 ± 1.08	6.13 ± 1.32	5.00 ± 0.90	6.24 ± 0.95	5.96 ± 1.16
Width [mm]	16.42 ± 2.07	16.17 ± 1.70	16.08 ± 1.38	15.17 ± 1.75	15.50 ± 1.68	16.00 ± 1.48
Thickness [mm]	3.46 ± 0.66	3.79 ± 0.58	4.54 ± 1.03	4.50 ± 0.85	4.00 ± 0.48	4.00 ± 0.64

length 5.91 ± 1.13 mm, width 15.89 ± 1.68 mm and thickness 4.05 ± 0.80 mm. Geometrical parameters of the specimens were measured using a Mitutoyo measuring system equipped with dial indicator accurate to 0.01 mm with adjustable pressing force. Table 1 shows the dimensions of the annulus fibrosus parameters for the corresponding sampling regions and segments. The sizes and orientations of all specimens were similar to eliminate these factors as confounding variables.

2.1. Hydration condition

Prior to the mechanical test, the specimens were subjected to hydration in 0.15% normal saline at room temperature. The aim of the hydration process was to restore proper concentration of water content in the test specimens of the annulus fibrosus of the intervertebral disc, which significantly affects the values of the mechanical parameters analysed [1], [11], [30].

Prior to placement in normal saline, the specimens were weighed on a Radwag PS 1000/C/2 precision balance with an accuracy of 0.001 g. Each specimen was then placed in a separate container with normal saline. The specimens were taken out of the saline at 10 minute intervals, weighed and again put in NaCl. The measurement was carried out for 60 minutes until the process stabilized. The increase of water content in the test specimen was determined from the difference between the dry sample weight and the hydrated sample weight.

Because the analysis of hydration was conducted for annulus fibrosus specimens with preserved bone attachments, tests were also carried out of a change in hydration for isolated fragments of the annulus fibrosus alone. This allowed us to determine the impact of bone parts and cartilaginous tissue, covering the surfaces of end plates of vertebral bodies, on the hydration process. To this end, a second group of specimens was prepared, obtained from a pig coming from the same breeder as the research material designated for mechanical tests. The specimens were prepared using the same procedure as in the case of the annulus fibrosus specimens with bone attachments. Then, using a scalpel, the annulus fibrosus was separated from the osteocartilaginous structure.

Analysis of the impact of hydration revealed that, regardless of the type of test specimens, the same correlation was obtained between the hydration curve and time. The first 30 minutes of immersion in normal saline showed much higher absorption of the solution in specimens with bone attachment, i.e., by 20% (n = 8) compared to specimens of the annulus fibrosus alone, where the value of the absorbed liquid increased by 9% (n = 8). Subsequent measurements showed no further significant increase of water in the specimens, hence the time required to restore physiological hydration should not be less than 30 minutes (Fig. 2). Additionally, for 6 selected specimens used in strength tests the impact of the study duration on a change in hydration was determined from the moment the specimen was taken out of saline to the end of the mechanical test. The total test time for a single specimen did not exceed 2 min. After this period, the observed decrease in the specimen weight associated with its dehydration averaged 4% of the weight obtained after 30 minutes of measurement.

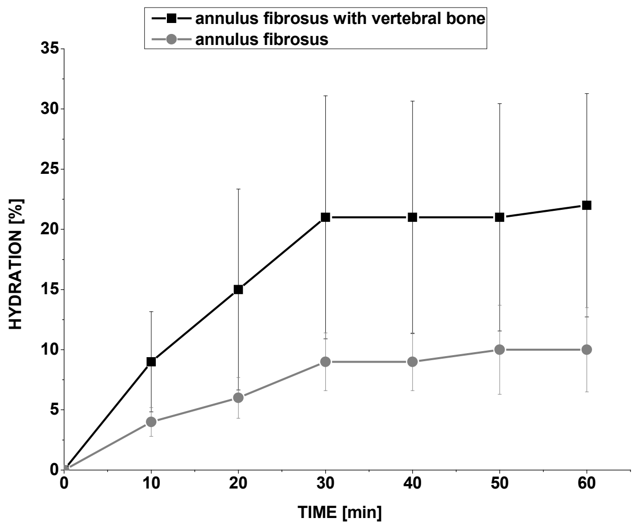

Fig. 2. Hydration changes with time for specimens of the annulus fibrosus (n = 8) and the annulus fibrosus with vertebral bone (n = 8) in NaCl saline environment. Analysis of the effect of hydration was carried out on 8 randomly selected specimens from the anterior part

2.2. Tensile testing

Bone parts of dissected specimens were mounted in special grips, whose surfaces were covered with coarse sandpaper, and then installed in an MTS Synergie 100 testing machine. The bone parts were inserted into a grip to half their length (i.e., to a length of about 5–7 mm of the total length of the bone part measuring about 15 mm), Fig. 3a. Each specimen was subjected to 5 initial tensile load cycles at a rate of 0.5 mm/s by application of a 1 mm displacement, which corresponds to about 20% of the maximum

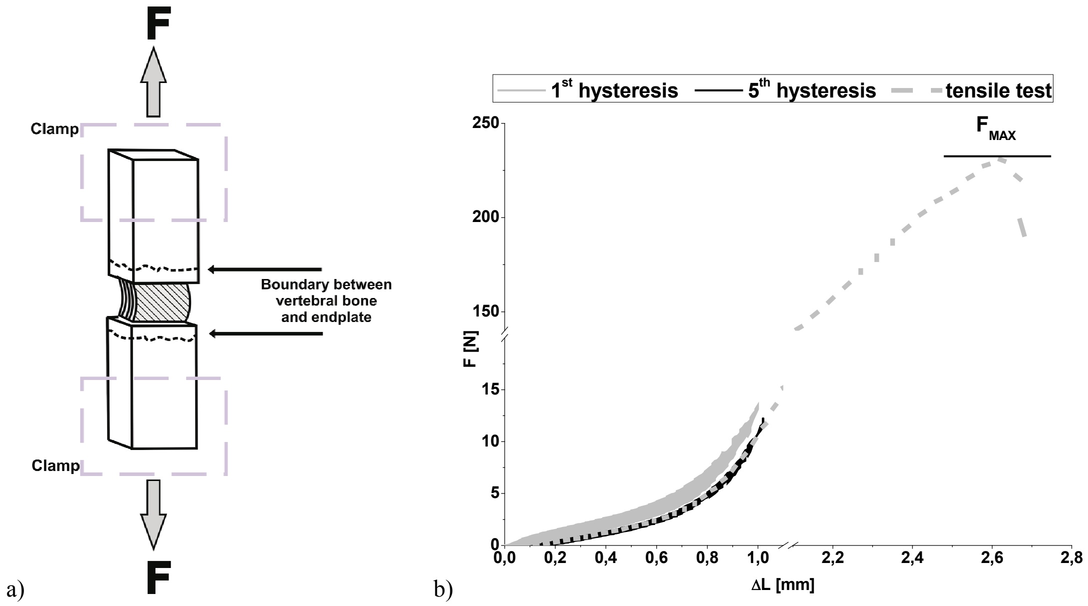

Fig. 3. Diagram of tensile loading of a specimen: (a) method of mounting a specimen in the grips, (b) sample force-displacement characteristics of a specimen pulled until fracture with indication of the breaking force range at the first and the fifth preload loop

value of strain, Fig. 3b [15], [5], [39]. Conditioning cycles were followed by uniaxial tensile loading of specimens at a constant rate of 0.5 mm/s until their fracture.

The obtained values of elongation of the specimen and the corresponding force values were used to determine characteristics of force as a function of displacement for consecutive hysteresis loops. On their basis the maximum force value was determined, obtained for a 1 mm displacement. On the basis of the resulting curves dissipated energy was determined for the first and fifth loop, defined as surface area limited by the maximum value of the force obtained during displacement of the specimen by a given value [23], [38].

Then, from the slope of the rectilinear section of the force-displacement and stress-strain curves (Fig. 4), obtained during the pulling of the specimen until its fracture, mechanical parameters were selected: stiffness (k) and Young's modulus (E). The values k and E were determined in the range from 35% to 70% of the value of maximum force (F_{MAX}) and ultimate tensile strength UTS (σ_{MAX}), which corresponds to the range in which we can see a significant share of collagen fibres in tensile loading [3]. The value of the conventional stiffness coefficient (k) is defined according to the formula

$$k = \text{tg}\alpha = \frac{F}{\Delta L}, \tag{1}$$

where: k [N/mm] – stiffness, α – slope of the curve $F(\Delta L)$, F [N] – force value, ΔL [mm] – elongation value.

2.3. Statistics

The results obtained were statistically analysed (OriginPro8 software) and presented as an average value together with standard deviations (X ± SD). Statistical analysis of the data was performed using the Student's t-test for independent tests. The tests were performed assuming that the limit value for the significance level was $p < 0.05$.

3. Results

The values of the force determined for a 1 mm displacement in successive loops of initial loading and unloading of specimens showed differences between the sampling areas (A, P) and between the individual segments of the spine (C, Th, L) (Table 2). At the same time, tests showed a significant drop in the value of the force in consecutive loops only in the thoracic spine. The biggest values of the force were obtained for the thoracic segment in the anterior part (48.61 ± 2.55 N), which were 24% higher than the values in the posterior part (37.11 ± 2.96 N). On the other hand,

Table 2. The range of the maximum force in subsequent hysteresis loops (n = 12).
Values are mean ±SD for all 3 spinal segments in the anterior and posterior locations of the annulus fibrosus

Spinal segments	Location of the annulus fibrosus	Number of cycle				
		1	2	3	4	5
		F_{MAX} [N]				
Cervical	anterior	2.36 ± 1.55	2.30 ± 1.56	2.28 ± 1.57	2.26 ± 1.57	2.26 ± 1.57
	posterior	1.04 ± 0.90	1.00 ± 0.84	0.97 ± 0.79	0.96 ± 0.78	0.96 ± 0.76
Thoracic	anterior	52.87 ± 17.41	48.77 ± 17.37	47.82 ± 17.54	47.22 ± 17.55	46.34 ± 17.17
	posterior	41.92 ± 13.01	37.83 ± 13.07	36.08 ± 12.92	35.25 ± 12.89	34.47 ± 13.26
Lumbar	anterior	18.94 ± 12.10	18.60 ± 11.88	18.45 ± 11.72	18.29 ± 11.72	18.22 ± 11.67
	posterior	29.45 ± 18.51	28.78 ± 17.40	28.35 ± 16.61	28.51 ± 16.69	28.06 ± 16.20

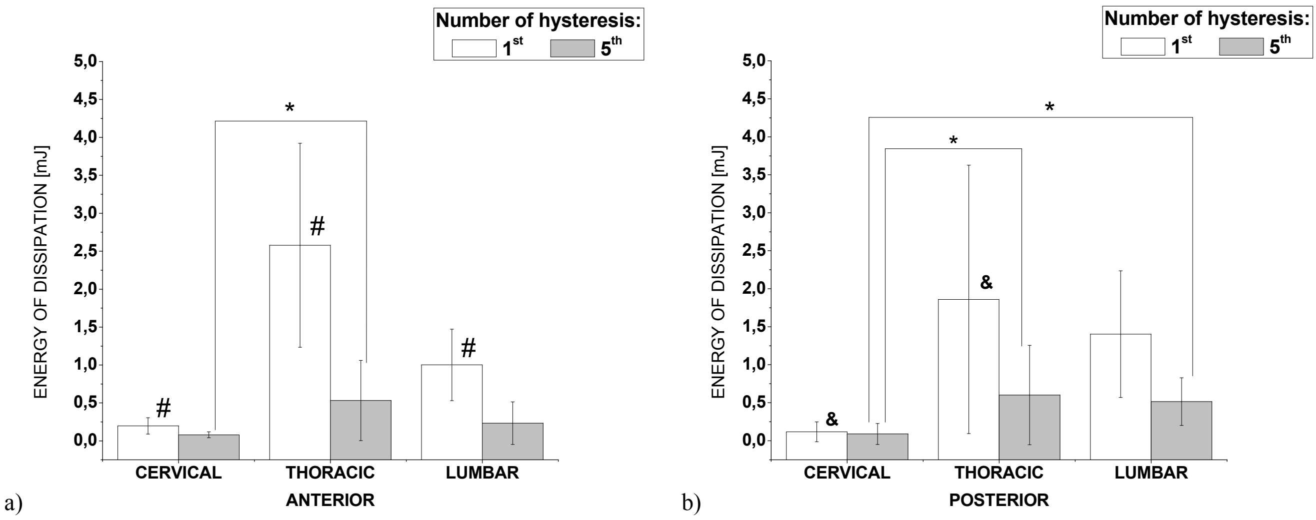

Fig. 4. Dissipated energy of subsequent hysteresis loops of initial loading and unloading of specimens in uniaxial tensile test (n = 9): (a) specimens from the anterior part; (b) specimens from the posterior part. * – statistically significant differences in dissipated energy in the last hysteresis ($p < 0.05$), # – statistically significant differences in dissipated energy in the first hysteresis between anterior margins of the C, Th and L motion segments ($p < 0.05$), & – statistically significant differences in dissipated energy in the first hysteresis between posterior margins of the C and Th motion segments ($p < 0.05$). In each of the A and P locations samples showed statistically significant differences between the first and the last hysteresis of ($p < 0.05$)

the smallest force was demonstrated by the annulus fibrosus in the cervical segment, whose value in the anterior part (2.29 ± 0.04 N) was 57% greater than the value obtained in the posterior part (0.99 ± 0.03 N). The forces obtained for posterior annuli from the lumbar spine (28.63 ± 0.53 N) were 35% higher than the values obtained in the anterior part (18.50 ± 0.29 N).

Hysteresis loops of initial loading and unloading are characterised by similar values of the maximum force in successive loops, where regardless of its value, a significant loss was observed of dissipated energy determined for the first and last (fifth) hysteresis loops. The biggest differences in values between the first and the fifth hysteresis loops were obtained for the thoracic segment, where the difference was 79% in part A and 68% in part P, and in the lumbar segment, where the difference was 77% in part A and 63% in part P. On the other hand, the smallest differences were obtained in the cervical segment, i.e., 59% in part A and 24% in part P. At the same time, the energy in the first loop of the thoracic segment (in part A – 2.58 ± 1.34 mJ and in part P – 1.86 ± 0.60 mJ) was statistically significantly higher compared to the values obtained in the cervical segment (Fig. 4a, b). The smallest loss of dissipated energy was obtained in part A of the cervical spine, whose value did not exceed 0.20 ± 0.08 mJ (in part A) and 0.17 ± 0.08 mJ (in part P) in the first hysteresis loop.

Analysis of individual mechanical parameters of the intervertebral disc showed statistically important differences between the sampling sites for anterior and posterior specimens (A, P). The value of UTS (σ_{MAX}) determined in the anterior part (A) was higher for the Th segment (4.70 ± 2.10 MPa) by 47% and for

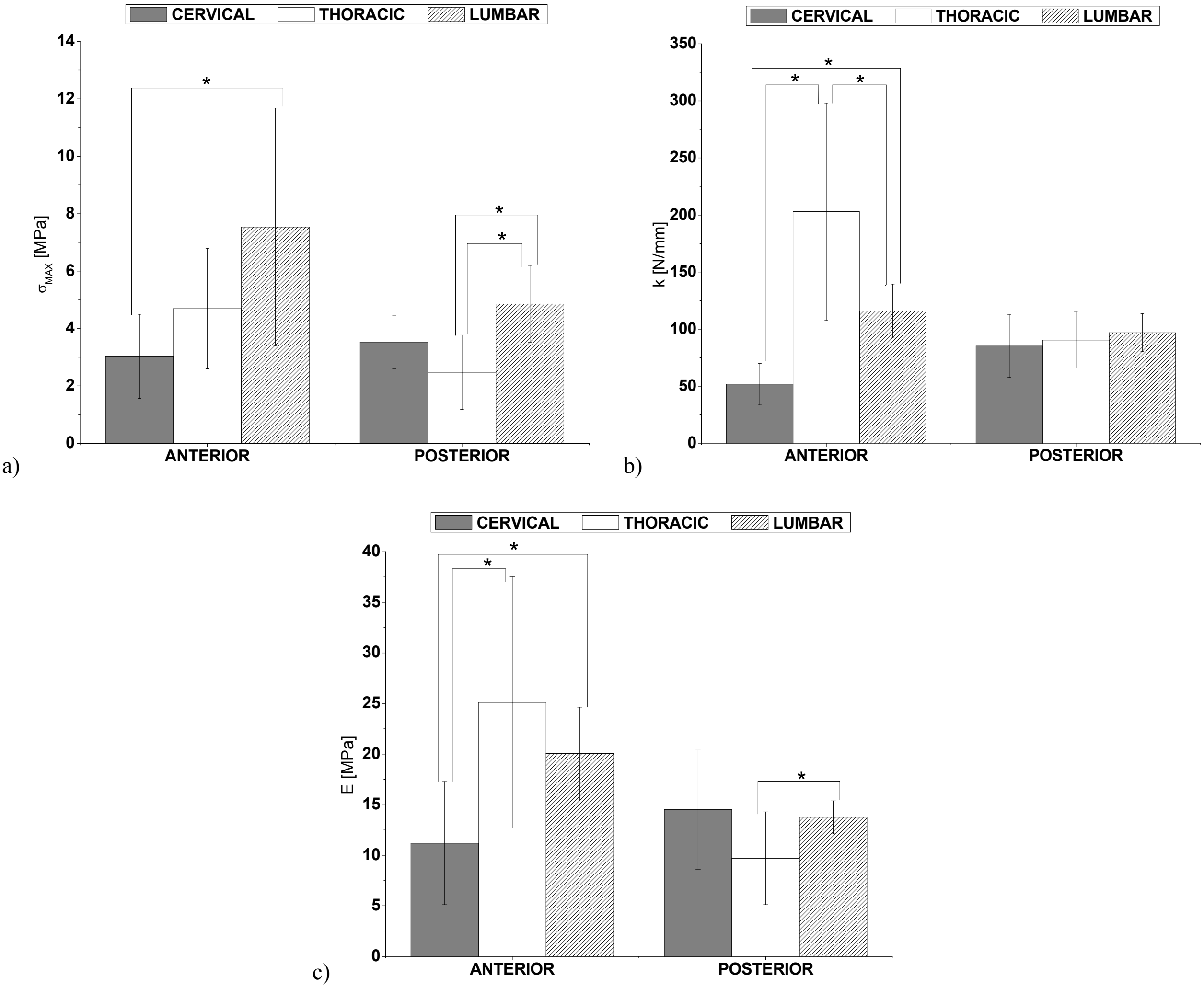

Fig. 5. The mechanical parameters of the annulus fibrosus together with bone attachment depending on various spinal segments collected from the anterior and posterior parts (n = 12): (a) ultimate tensile strength – UTS (σ_{MAX}); (b) the stiffness value (k); (c) Young's modulus value (E). * – Statistically significant differences between 3 segments of the spine in anterior and posterior locations of the annulus fibrosus in intervertebral disc segments ($p < 0.05$)

the L segment (7.54 ± 4.14 MPa) by 36% compared to part P, where it amounted to 2.47 ± 1.29 MPa for the thoracic segment and 4.86 ± 1.36 MPa for the lumbar segment. At the same time, the value of UTS of the annulus fibrosus in the anterior part increases as the spinal segment changes (C-Th-L). The smallest values, with the level of statistical significance of 0.05, were obtained in the cervical segment (3.03 ± 1.47 MPa) compared to the lumbar segment (7.54 ± 4.14 MPa) – Fig. 5a.

On the other hand, the value of the conventional stiffness coefficient (k), shown in Fig. 5b, indicates that there are significant differences in part A. The highest stiffness coefficient was demonstrated by the Th segment (203.00 ± 95.913 N/mm), which was higher by 43% compared to the L segment (115.85 ± 23.56 N/mm) and higher by 74% compared to the cervical segment (51.80 ± 18.22 N/mm).

The highest values of the conventional Young's modulus (E) were observed in the anterior part (A) for the thoracic (25.11 ± 12.40 MPa) and lumbar (20.05 ± 4.58 MPa) segments, which were higher than the values obtained in the posterior part by 61% for the Th segment (9.69 ± 4.58 MPa) and by 31% for the *L* segment (13.75 ± 1.63 MPa) – Fig. 5c.

4. Discussion

This work determined the mechanical parameters of multilayer specimens of the annulus fibrosus in a uniaxial tensile test according to the sampling site (anterior and posterior) and the spinal segment (cervical, thoracic and lumbar) from which the research material was sampled.

The annulus fibrosus exhibits viscoelastic properties [5], which means that during deformation stress and strain are out of phase. During loading and subsequent unloading, a certain strain delay can be observed in relation to the acting stress and, consequently, the appearance of a hysteresis loop. The area of the hysteresis loop obtained shows dissipated energy, which is defined as a mechanical parameter that makes it possible to characterize nonlinear mechanical properties of soft tissues [17], [40]. Based on initial loading and unloading of annulus fibrosus specimens studied in the above work, an assessment was made of the quantitative losses of dissipated energy arising in successive hysteresis loops.

Analysis of dissipated energy in each of the five load loops showed big changes in subsequent cycles compared to the value of the force obtained under the same loading conditions (Table 2). These changes take the form of continued energy loss with simultaneously decreasing dynamics of this process. If we compare the structure of the annulus fibrosus to the structure of a fibrous multilayer composite [24], then, as demonstrated by Porębska et al. [26], the higher the fibre content in the composite material, the higher the value of dissipated energy. At the same time, random distribution of the fibres favours formation of areas with locally higher stress values, in which a decrease in the value of dissipated energy in subsequent loops results from fracture of adhesive connections between the matrix and the coating as well as within fibres [20], [26].

The studies conducted showed no statistically important differences in the obtained energy values between the anterior and posterior parts of the respective spinal segments, which is consistent with the observations of the authors [11]. The highest differences in dissipated energy at precondition were obtained for annulus fibrosus specimens from the thoracic and lumbar segments. In the cervical segment the recorded energy losses were negligible (in the range of 0.08÷0.16 mJ) compared to the obtained ranges for the thoracic (0.5÷2.3 mJ) and lumbar (0.3÷1.2 mJ) segments. On the other hand, statistically significant changes (regardless of parts A, P) were observed between the first and fifth loops of initial loading for all specimens analysed.

The resulting differences in the values of dissipated energy between the first and last hysteresis loops reflect work in the structure of the annulus fibrosus matrix, i.e., the work used to spread out and move crimp collagen fibres as well as the work between the fibres and the coating consisting of proteoglycans. The resulting losses of dissipated energy may also result from microcracks of collagen fibres forming connections between annulus lamellae during loading.

Introduction of initial cycle loading and unloading minimizes the impact of viscoelastic properties of the tissue thereby restoring its actual biomechanical properties [37].

In parallel with the analysis of dissipated energy, the maximum values of the force for the precondition test were also determined. Differences in the recorded force were observed depending on the tested segment of the spine and the site from which the annulus fibrosus was sampled. Also, as in the case of dissipated energy, the highest values of strength were demonstrated by specimens from the thoracic and lumbar segments while the smallest values were exhibited by specimens from the cervical segment (Table 2). Also, higher strength values were recorded for anterior fragments of annulus fibrosus. However, the most important observation in this part of the analysis was demonstration of the lack of significant changes in the value of strength in successive conditioning loops. This clearly indicates that the parameter of maximum strength determined with regard to elastic loads does not allow description of changes taking place during the work of the intervertebral disc under the impact of physiological loads.

During the second stage of the research, the samples subjected to initial loading were tensile tested until fracture. Significantly, it was demonstrated that the values of Young's modulus and ultimate tensile strength (UTS) are influenced by both the spinal segment used to collect the specimen and by the sampling site in the annulus itself (anterior/posterior site margins).

The average UTS value (σ_{MAX}) in the anterior part was 5.09 ± 3.30 MPa and in the posterior part it was (P) 3.62 ± 1.52 MPa, which is consistent with the results presented in the study [11]. In the case of tests of human spine specimens, the UTS values of the outer part of the annulus fibrosus with bone attachment averaged 1.5÷4.0 MPa [15] and without attachments 10.3 ± 8.4 MPa [30] and were twofold higher than in the posterior part. Statistically significant differences in the values of the stiffness coefficient and Young's modulus among the analysed segments of the spine (C, Th, L) occur primarily in the anterior part of the intervertebral disc. Specifically, the average values of the stiffness coefficient in the anterior part were greater by 43% compared to the posterior part. The same relationship occurred in the case of Young's modulus, for which the difference in mean values between the anterior (18.79 ± 7.04 MPa) and posterior (12.65 ± 2.59 MPa) parts was 33%.

The results obtained indicate that distribution of lamellae in the annulus fibrosus is heterogeneous. Assuming the same specimen thickness, there is a different number of lamellae of the annulus fibrosus depending on the anatomical region of intervertebral disc. Compared to lamellae in the anterior part, posterior annuli are characterised by thinner lamellae (especially in outer layers) and the presence of incomplete layers [9], [21]. Strong differentiation in the hierarchical arrangement of the annulus fibrosus of the anterior part in relation to the posterior part affects the strength parameters of this structure. Consequently, in terms of strength the posterior margin of the intervertebral disc is weaker and quicker to destroy. These observations are consistent with clinical observations, which most often show damage to the intervertebral disc in its posterior part [2], [7], [22], [28], [29], [35].

Statistically significant spread of values of the mechanical parameters analysed in this study is due to the anatomical differentiation of structures of the spine in its respective segments. This is primarily due to the distribution of the transferred loads and the range of motion attributable to the respective segments of the spine. This is especially visible in the anterior part, for which the average value of the mechanical parameters in the cervical segment is smaller and increases with subsequent segments of the spine.

The obtained results were influenced to a large degree by the fact that the tests were carried out on specimens whose annulus fibrosus was anchored in the end plate. Therefore, distribution of the axial force acting on collagen fibre bundles was heterogeneous in individual lamellae but corresponded to physiological states existing in the intervertebral disc.

From the viewpoint of description of the test material characterised by strong hydration, an important element of the study undertaken was an analysis of the state and dynamics of hydration of the specimens of annulus fibrosus prior to and during strength testing. Restoration of physiological hydration of the intervertebral disc before the final strength test eliminates the impact of swelling on the values obtained of mechanical parameters of the tissue. This fact was repeatedly emphasized in the works of Ebara and Costi [10], [11]. The geometric changes of the structure of the annulus fibrosus caused by swelling substantially affect the mechanical parameters determined. Therefore, one of the aims of the research was to characterize the process of hydration of annulus fibrosus specimens. The evaluation concerned the course of swelling and the impact of the presence of osteocartilaginous elements on absorption of water by the intervertebral disc. The disturbance of the processes of osmotic flow of fluids between the connections of the structures of the intervertebral disc and the end plane affects a drop in the water content in the intervertebral disc, accelerating the formation of degenerative changes in its structure. Hydration had the greatest impact on an increase in the thickness of the specimen, enhancing it by an average of 1.49±0.45 mm. Similar to a study by Skaggs et al. [30], a change was observed in the surface area of the element tested (annulus fibrosus) during hydration. Regardless of the presence of bone elements, both groups of the samples showed that the time required to restore full saturation with the NaCl solution should not be less than 30 minutes. After this time, both specimens with vertebral body fragments and the annulus fibrosus itself showed no further increase in water. As shown by studies conducted by Acaroglu and Ebara, this time is also sufficient for full hydration of the outer and inner layers of the annulus fibrosus [1, 11].

The results obtained clearly indicate a wide differentiation in the mechanical properties among the anatomical regions and in the spine segments.

Conclusion

1. Dissipated energy is a parameter enabling description of the work of annulus fibrosus of the intervertebral disc with regard to the impact of physiological loads.

2. Significant differences were shown in the values of dissipated energy between the first and last preload loops. Heterogeneous distribution of lamellae in the annulus fibrosus (anterior/posterior) does not determine higher energy losses with regard to elastic loads.

3. Young's modulus and ultimate tensile strength show a strong correlation with the spine part and IVD region.

4. In order to obtain equilibrium in the hydration of the annulus fibrosus, the swelling process should not take less than 30 minutes.

Acknowledgements

This work was supported by the grant N N518501139.

References

[1] ACAROGLU E.R., IATRIDIS J.C., SETTON L.A., FOSTER R.J., MOW V.C., WEIDENBAUM M., *Degeneration and aging affect the tensile behavior of human lumbar anulus fibrosus*, Spine, 1995, 20(24), 2690–2701.

[2] ADAMS M., DOLAN P., *Spine biomechanics*, J. Biomech., 2005, 38, 1972–1983.

[3] ADAMS M.A., GREEN T.P., *Tensile properties of the annulus fibrosus. I. The contribution of fiber-matrix interactions to tensile stiffness and strength*, Eur. Spine J., 1993, 2, 203–208.

[4] ALINI M., EISENSTEIN S.M., ITO K., LITTLE C., KETTLER A.A., MASUDA K., MELROSE J., RALPHS J., STOKES I., WILKE H.J., *Are animal models useful for studying human disc disorders/degeneration?*, Eur. Spine J., 2007, 17, 1, 2–19.

[5] AMBARD D., CHERBLANC F., *Mechanical behavior of annulus fibrosus: a microstructural model of fibers reorientation*, Ann. Biomed. Eng., 2009, 37(11), 2256–2265.

[6] BASS E.C., ASHFORD F.A., SEGAL M.R., LOTZ J.C., *Biaxial testing of human annulus fibrosus and its implications for a constitutive formulation*, Ann. Biomed. Eng., 2004, 32(9), 1231–1242.

[7] BEATTIE P., *Current Understanding of Lumbar Intervertebral Disc Degeneration: A Review With Emphasis Upon Etiology*, J. Orthop. Sports Phys. Ther., 2008, 38(6), 329–340.

[8] BECKSTEIN J.C., SEN S., SCHAER T.P., VRESILOVIC E.J., ELLIOTT D.M., *Comparison of animal discs used in disc research to human lumbar disc axial compression mechanics and glycosaminoglycan content*, Spine, 2008, 33(6), 166–173.

[9] CASSIDY J.J., HILTNER A., BAER E., *Hierarchical structure of the intervertebral disc*, Connective Tissue Res., 1989, 23, 75–88.

[10] COSTI J.J., HEARN T.C., FAZZALARI N.L., *The effect of hydration on the stiffness of intervertebral discs in an ovine model*, Clin. Biomech., 2002, 17, 446–455.

[11] EBARA S., IATRIDIS J.C., SETTON L.A., FOSTER R.J., MOW V.C., WEIDENBAUM M., *Tensile properties of nondegenerate human lumbar anulus fibrosus*, Spine, 1996, 21(4), 452–461.

[12] ELLIOTT D.M., SETTON L.A., *Anisotropic and inhomogeneous tensile behaviour of the human annulus fibrosus: experimental measurements and material model predictions*, J. Biomech. Eng., 2001, 123, 256–263.

[13] FUJITA Y., DUNCAN N.E., LOTZ J.C., *Radial tensile properties of the lumbar annulus fibrosis are site and degeneration dependent*, J. Orthop. Res., 1997, 15, 814–819.

[14] GALANTE J.O., *Tensile properties of the human lumbar annulus fibrosus*, Acta Orthop. Scand., 1967, Suppl. 100, 1–91.

[15] GREEN T.P., ADAMS M.A., DOLAN P., *Tensile properties of the annulus fibrosus. II. Ultimate tensile strength and fatigue life*, Eur. Spine J., 1993, 2, 209–214.

[16] HOLZAPFEL G.A., SCHULZE-BAUER C.A.J., FEIGL G., REGITNIG P., *Single lamellar mechanics of the human lumbar anulus fibrosus*, Biomech. Model. Mechanobiol., 2005, 3, 125–140.

[17] HSU C.C., TSAI W.C., SHAU Y.W., LEE K.L., HU C.F., *Altered energy dissipation ratio of the plantar soft tissues under the metatarsal heads in patients with type 2 diabetes mellitus: a pilot study*, Clin. Biomech., 2007, 22, 67–73.

[18] HUYGHE J.M., *Biaxial testing of canine annulus fibrosus tissue under changing salt concentrations*, An. Acad. Bras. Cienc., 2010, 82(1), 145–151.

[19] INOUE H., *Three-dimensional architecture of lumbar intervertebral discs*, Spine, 1981, 6(2), 139–146.

[20] KATUNIN A., *The conception of the fatigue model for layered composites considering thermal effects*, ACME, 2011, 11(2), 333–343.

[21] MARCHAND F., AHMED A.M., *Investigation of the laminate structure of lumbar disc anulus fibrosus*, Spine, 1990, 15(5), 402–410.

[22] MISTERSKA E., JANKOWSKI R., GŁOWACKI M., *Quebec Back Pain Disability Scale, Low Back Ouctome Score and Revised Oswestry Low Back Pain Disability Scale for Patients with Low Back Pain due to degenerative disc disease*, Spine, 2011, 36(26), E 1722–1729.

[23] NUCKLEY D.J., KRAMER P.A., DEL ROSARIO A., FABRO N., BARAN S.Z., CHING R.P., *Intervertebral disc degeneration in a naturally occurring primate model: radiographic and biomedical evidence*, J. Orthop. Res., 2008, 26, 1283–1288.

[24] PEZOWICZ C., *Analysis of selected mechanical properties of intervertebral disc annulus fibrosus in macro and microscopic scale*, Journal of Theoretical and Applied Mechanics, 2010, 48(4), 917–932.

[25] PEZOWICZ C.A., ROBERTSON P.A., BROOM N.D., *Intralamellar relationships within the collagenous architecture of the annulus fibrosus imaged in its fully hydrated state*, J Anat., 2005, 207(4), 299–312.

[26] PORĘBSKA R., MAZURKIEWICZ S., *Dissipated energy in polimeric composites*, (in Polish), Czasopismo Techniczne Politechniki Krakowskiej, 2009, 3, 245–249.

[27] RODRIGUES S.A., WADE K.R., THAMBYAH A., BROOM N.D., *Micromechanics of annulus-end plate integration in the intervertebral disc*, Spine J., 2012, 12(2), 143–150.

[28] SCHULTZ A.B., ASHTON-MILLER J.A., *Biomechanics of the human spine*, [in:] Van C. Mow, W.C. Hayes (eds.), Basic Orthopeadic Biomechanics, Raven Press, 1991, 337–374.

[29] SHANKAR H., SCARLETT J., ABRAM S.E., *Anatomy and pathophysiology of intervertebral disc disease*, Tech. Reg. Anesth. Pain Manag., 2009, 13, 67–75.

[30] SKAGGS D.L., WEIDENBAUM M., IATRIDIS J.C., RATCLIFFE A., MOW V.C., *Regional variation in tensile properties and biochemical composition of the human lumbar anulus fibrosus*, Spine, 1994, 19(12), 1310–1319.

[31] SMIT T.H., *The use of a quadruped as an in vivo model for the study of the spine – biomechanical considerations*, Eur. Spine J., 2002, 11, 137–144.

[32] SZOTEK S., SZUST A., PEZOWICZ C., MAJCHER P., BĘDZIŃSKI R., *Animal models in biomechanical spine investigations*, Bull. Vet. Inst. Puławy, 2004, 48(2), 163–168.

[33] TSUJI H., HIRANO N., OHSHIMA H., ISHIHARA H., TERAHATA N., MOTOE T., *Structural variation of the anterior and posterior annulus fibrosis in the development of human lumbar intervertebral discs: a risk factor for intervertebral disc rupture*, Spine, 1993, 18, 204–210.

[34] WADE K.R., ROBERTSON P.A., BROOM N.D., *A fresh look at the nucleus-endplate region: new evidence for significant structural integration*, Eur. Spine J., 2011, 20(8), 1225–1232.

[35] WANG Y., CHEN H.B., ZHANG L., ZHANG L.Y., LIU J.C., WANG Z.G., *Influence of degenerative changes of intervertebral disc on its material properties and pathology*, Chin. J. Traumatol., 2012, 15(2), 67–76.

[36] WHITE A.A., PANJABI M.M., *Clinical biomechanics of the spine*, Lippincott (Philadelphia), 1990.

[37] WILKE H.J., WENGER K., CLAES L., *Testing criteria for spinal implants: recommendations for the standardization of in vitro stability testing of spinal implants*, Eur. Spine J., 1998, 7, 148–154.

[38] ŻAK M., *Dissipated energy in annulus fibrosus of intervertebral disc*, (in Polish), Aktualne Problemy Biomechaniki, 2010, 4, 285–288.

[39] ŻAK M., KUROPKA P., KOBIELARZ M., DUDEK A., KALETA-KURATEWICZ K., SZOTEK S., *Determination of the mechanical properties of the skin of pig foetuses with respect to its structure*, Acta Bioeng. Biomech., 2011, 13(2), 38–43.

[40] ŻAK M., PEZOWICZ C., *The energy dissipation of multilamellar annulus fibrosus of intervertebral disc*, Journal of Biomechanics, 2012, 45 Supplement 1, S573.