

## **ANALYSIS OF SELECTED MECHANICAL PROPERTIES OF INTERVERTEBRAL DISC ANNULUS FIBROSUS IN MACRO AND MICROSCOPIC SCALE**

CELINA PEZOWICZ

*Wroclaw University of Technology, Institute of Machine Design and Operation, Poland*

*e-mail: celina.pezowicz@pwr.wroc.pl*

The main goal of this paper is experimental analysis of selected mechanical properties of single annulus fibrosus lamellae at macroscopic and microscopic levels as well as representation and explanation of the structural response to forced deformation. The conducted analysis of single annulus fibrosus lamella with preserved natural attachments revealed two characteristic mechanisms leading to damage of annulus fibrosus and large diversification of conventional Young's modulus. The analysis in a microscopic level enable the imaging of changes in the collagen matrix during a tensile test and of significant differences in mechanical properties with respect to tensile direction.

*Key words:* spine, intervertebral disc, tensile test, structural configuration

### **1. Introduction**

The ability of the human spine to transfer variable loads and perform a broad range of motion is possible thanks to the complex structure and function of intervertebral discs which, together with vertebrae, are the basic components of the spine.

A key element for the proper functioning of the spine is the intervertebral disc, which plays an important role in the transfer of loads and amortisation of the spine, as demonstrated by early works of Hirsch and Nachemson (1954), Nachemson (1960).

An intervertebral disc consists of annulus fibrosus and nucleus pulposus. An important role in proper operation of the disc is played by annulus fibrosus located externally and made of 15-25 adjoining, concentric layers – lamellae (Marchand and Ahmed, 1990; Cassisy *et al.*, 1989). The lamellae are made

of collagen fibres, lying parallel to each other and tilted at an angle of  $30^\circ$  (the internal lamellae are tilted by as much as  $\sim 45^\circ$ ). The fibres of successive lamellae are arranged alternately and intersect with each other (right/left-hand alignment).

Proper functioning of annulus fibrosus determines correct work of the intervertebral disc, and thus the entire spine. Annulus fibrosus is predominantly subjected to a tensile force in the course of its physiological activities. The above force is a result of compression load, which reduces the height of the whole intervertebral disc by bulging of annuli fibrosus to the external part of the disc.

The functioning of annulus fibrosus is closely related to its internal architecture, especially the organisation of collagen fibres. Deformations of a single fibre or a bundle of collagen fibres determine nonlinear, strongly anisotropic mechanical properties of the whole annulus fibrosus.

The complex, multi-layer architecture of annulus fibrosus still presents an enormous research challenge. There is an ongoing search for data which would provide a deeper understanding of the fundamental structural relationship of annulus fibrosus, which enables transfer of variable values of loads appearing in the spine during normal life functions as well as overload conditions.

In a macroscopic scale, strength tests of annulus fibrosus are conducted for both isolated, single lamellae as well as bundles of lamellae. The main focus is placed on examination of mechanical properties in a uniaxial tensile test depending on such parameters as: donor age, degree of tissue degeneration, sampling site (anterior or posterior part of annulus fibrosus), or tensile direction (longitudinal/transverse) (Galante, 1967; Wu and Yao, 1976; Adams and Green, 1993; Green *et al.*, 1993; Skaggs *et al.*, 1994; Acaroglu *et al.*, 1995; Ebara *et al.*, 1996; Fujuta *et al.*, 1997; Eliott and Setton, 2001). Tests of mechanical properties of the annulus fibrosus structure are conducted most often on isolated, multilayer samples (consisting of between a few and more than a dozen annulus fibrosus lamellae) (Adams and Green, 1993) or on fragments of single lamellae (Holzapfel *et al.*, 2005). However, there are no analyses which would involve strength tests of a single annular wall with preserved natural attachments on vertebral bodies.

Tests of material parameters of fibrous annular tissue without simultaneous analysis of the changes taking place in its structure do not provide sufficient information on the factors determining the obtained values of the analysed mechanical parameters. An analysis of the structural changes in annulus fibrosus with simultaneous examination of its mechanical properties on the microscopic level is carried out only rarely. In this area, only Bruehlmann *et al.* (2004)

looked at changes in intercellular and collagen network arrangement subjected to bending stress. In addition to tests of mechanical properties, additional insight could be obtained on a microscopic level into changes occurring in the collagenous matrix configuration to explain many mechanisms governing the performance characteristics and the properties of the intervertebral disc.

Therefore, tests were undertaken to analyse selected mechanical properties of single annulus fibrosus lamellae at macroscopic and microscopic levels as well as to represent and explain the structural response to forced deformation.

## 2. Materials and methods

For the purposes of conducted tests, the macroscopic system is a system containing a single annulus fibrosus lamella with a diameter of 0.2 mm with preserved natural attachments. On the other hand, a microscopic system consists of samples of a single annulus fibrosus lamella with the thickness of the order of  $\mu\text{m}$ , enabling simultaneous analysis of mechanical properties and visualisation of structural changes.

### 2.1. Testing of mechanical properties of a single annulus fibrosus lamella on macroscopic scale

The tests were conducted on animal intervertebral discs. In order to obtain a single annulus fibrosus lamella, soft tissue was cleaned off the motor segment of the spine until the merger of vertebral bodies with the intervertebral disc. Next, along collagen fibres of the annulus (running from bone to bone), a block was cut out containing several annulus fibrosus lamellae with bone fragments of vertebral bodies. During the last stage of preparation of the samples, a single annulus fibrosus lamella was obtained by gentle separation of the lamella and removal of the remaining ones – Fig. 1a. In the end, 17 samples were obtained of a single annular fibrosus wall with preserved natural attachments to the bone tissue of the vertebral body – Fig. 1b. The dimensions of the samples were normalised. The samples had the average length of  $14.47 \pm 2.89$  mm, width of  $4.12 \pm 0.92$  mm, and thickness of  $0.20 \pm 0.14$  mm.

The prepared research material was subjected to a uniaxial tensile test in the direction of the alignment of collagen fibres – Fig. 1b. For this purpose, the sample was fastened to mounting clamps through the top and bottom parts of bone tissue and attached directly to the material testing machine MTS Synergie 100.

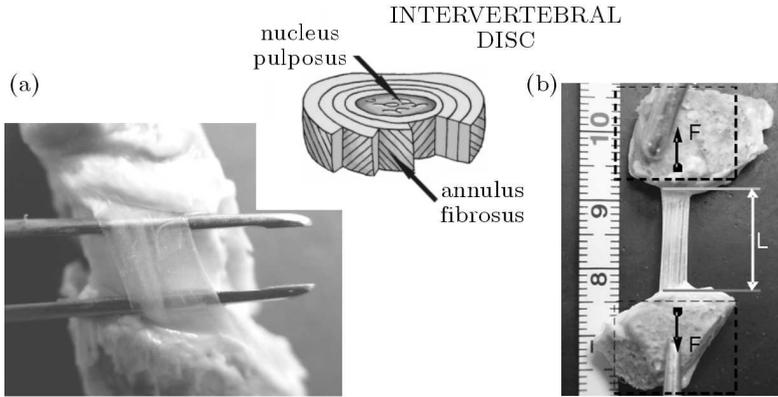


Fig. 1. Photographs showing: (a) separation of a single annular lamella, (b) final sample containing a single annulus fibrosus lamella with attachments in vertebral bodies ( $F$  – tensile force,  $L$  – total sample length)

In order to reduce the effect of loss of moisture of the tested tissue material, the samples remained in 0.15% normal saline (30 min) before the start of test (Skaggs *et al.*, 1994).

The strength of the annulus fibrosus structure was tested in the tensile test during which the force changes were recorded as a function of displacement.

The sample was subjected to initial stretching, in which the length increase equalled approx. 5% of the sample length. For each sample, three initial tension loops were carried out at a rate of 2 mm/min, followed directly by tensile stretching until the time of rupture (rate of tensile extension equalled 2 mm/min).

## 2.2. Testing of mechanical and structural properties of a single annulus fibrosus lamella in microscopic scale

The tests were conducted on animal intervertebral discs. Before the testing began, single motor segments were cut out of the whole frozen spinal segment, which were then defrosted and cleaned off soft tissue and discs to obtain an isolated intervertebral disc. From the intervertebral discs prepared in the above way, blocks of external part of annulus fibrosus were cut out, which were then frozen in liquid nitrogen and finally sliced at a thickness of 50-60  $\mu\text{m}$  – Fig. 2. As a result of cutting, samples were obtained containing single annulus fibrosus lamellae with a uniform, parallel arrangement of collagen fibres (Pezowicz *et al.*, 2005).

The structure of the obtained micro-samples was visualised with the use of an interference light microscope. Analysis of the structural changes was carried

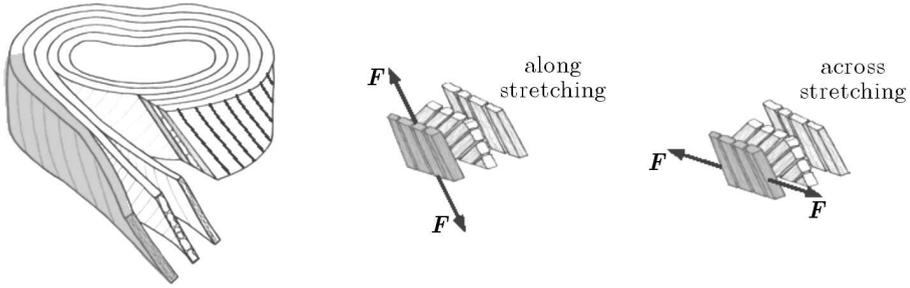


Fig. 2. A diagram showing the methodology of obtaining samples of a single annulus fibrosus lamella and their tensile directions

out during the stretching of samples in a special stretching set-up (Broom, 1984,1986) attached directly to the revolving table of the light microscope. The applied rate of tensile extension was 0.4 mm/min.

During the tests, changes of force were recorded as a function of displacement with the simultaneous analysis of the structural changes taking place in the sample during stretching. The tensile strain was determined as a strain coefficient  $\lambda$ , expressing the relationship of post-stretching length to the output length of the tested sample (Pezowicz *et al.*, 2005).

### 3. Results

#### 3.1. Tensile test in macroscopic configuration

Tensile tests involving single lamella with preserved bone attachments produced tensile force values as a function of displacement (increase of sample length). The relationship between the force and displacement was then used to determine the tensile stress as a function of sample strain.

The conducted analysis revealed two characteristic mechanisms leading to damage of annulus fibrosus. The first damage mechanism is represented by samples in which the maximum force during tensile stretching reached an average value of  $41.9 \pm 7.9$  N ( $n = 7$ ), above which there was a sharp drop in relation to displacement. In the second mechanism, damage occurred mostly in the region of the upper bone attachment with the accompanying dissections in the upper part of the sample while the average force of damage amounted to  $12,3 \pm 3.4$  N ( $n = 10$ ). The sample recorded in function of displacement for the first and second rupture mechanism is presented in Fig. 3.

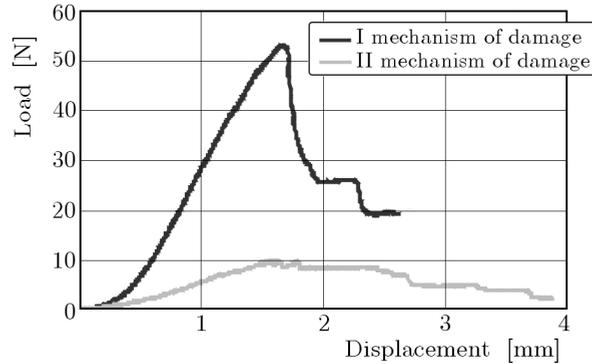


Fig. 3. Exemplary characteristics of changes in the tensile force as a function of displacement for the first and second rapture mechanism

In order to determine conventional Young's modulus, the stress-strain characteristics were divided into three equal ranges (lower, medium, and upper) on a scale from zero to the maximum stress. Those ranges were searched for linear regions, which were used to determine the direction component (Fig. 4). Although the characteristics in the first rapture mechanism were practically linear in the whole range of stress increase, they were also divided into the three regions.

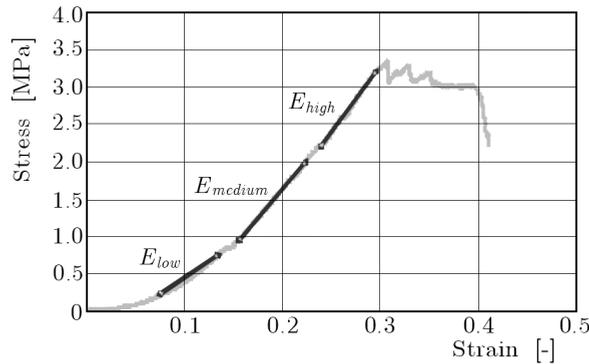


Fig. 4. Characteristics of tensile stress as a function of deformation with marked ranges of Young's modulus analysis

Table 1 shows values of Young's modulus for respective sections of the stress-strain curve depending on the damage mechanism. In the case of both the first and the second damage mechanisms, the most important values of the modulus were obtained in the medium part of the curve.

**Table 1.** Conventional Young's modulus [MPa] in the respective ranges

	$E_{low}$	$E_{medium}$	$E_{high}$
1st mechanism	$39.3 \pm 5.0$	$46.9 \pm 8.1$	$35.5 \pm 8.0$
2nd mechanism	$37.2 \pm 3.2$	$45.7 \pm 2.4$	$31.1 \pm 3.6$

### 3.2. Tensile test in microscopic configuration

During the first stage, the analysis covered structural changes and selected mechanical properties occurring during longitudinal tension (in accordance with the arrangement of collagen fibres). As a result of the conducted tests, a series of photographs was obtained for the respective stages of tensile stretching as well as the stress-strain characteristics corresponding to the simultaneously recorded structural changes.

Figure 5 shows typical stress-strain characteristics, obtained during stretching of peripheral samples in the longitudinal direction – along stretching (in the direction of alignment of collagen fibres) and in the direction transverse to the alignment of collagen fibres of a single annulus fibrosus lamella – across stretching.

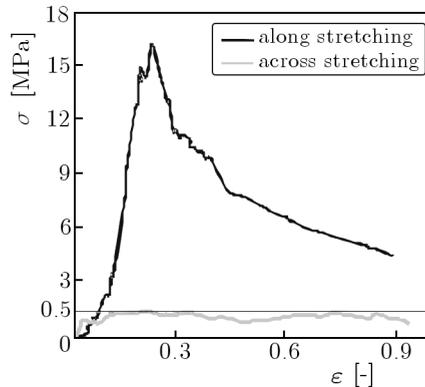


Fig. 5. Sample stress-strain characteristics of a single annulus fibrosus lamella subjected to stretching

In the initial phase, the crimp structure of collagen fibres (Fig. 6a) is progressively straightened and tightened. A reduced tension of the sample in this range of the stress-strain curve results in a return of the crimp nature of collagen fibres. The maximum average values of tension equalled  $\sigma_{max} = 16.4 \pm 8$  MPa, where the obtaining of the maximum tension value signalled the commencement of a large-scale rupture process along the entire length of the sample. The rapidly declining stress corresponds to the progres-

sive increase in displacement and rupture of collagen fibres throughout the area (matrix) of the sample. At the same time, the maximum value of stress in Fig. 5 marks the commencement of the reduced stress region resulting from the large-scale separation of collagen fibres (Fig. 6b).

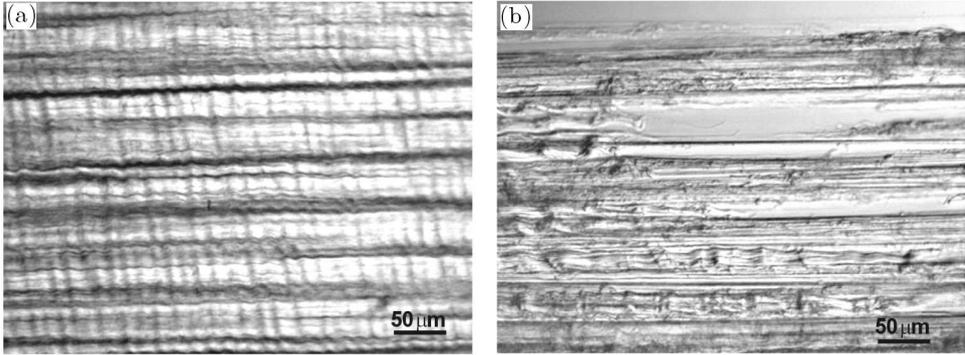


Fig. 6. Photographs of a single annulus fibrosus lamella: (a) in the unloaded state with distinctive crimp structure, (b) showing separation of collagen fibres during tensile stretching along the fibre alignment direction

Also note that even single collagen fibres, following their detachment, tend to return to the characteristic pre-load crimp.

During the second stage, the analysis covered structural changes and selected mechanical properties occurring during transverse stretching (transverse to the collagen alignment direction).

The final result of such stretching is the state showing extensive separation of collagen fibres in the middle part from the almost unaffected structure of the collagen matrix with the maintained parallel alignment of fibres. Also, during such intensive rearrangement of the collagen structure, there is an almost constant level of stress (Fig. 5). For a constant rate of tensile extension (0.4 mm/min), all tested samples revealed an average stress value of  $0.15 \pm 0.06$  MPa.

By recording the changes taking place from the state unloaded through the subsequent stretching phases, an analysis of the reorganisation mechanism of the structure of annulus fibrosus lamella is possible. The aligned fibres begin to separate in isolated regions – clefts, exposing a network of collagenous intersections that cross obliquely from both sides. With increased stretching, these same clefts open even further, accompanied by extensive splitting and skewing of the still intact fibre bundles. At the same time, new clefts are created in further regions of the stretched sample.

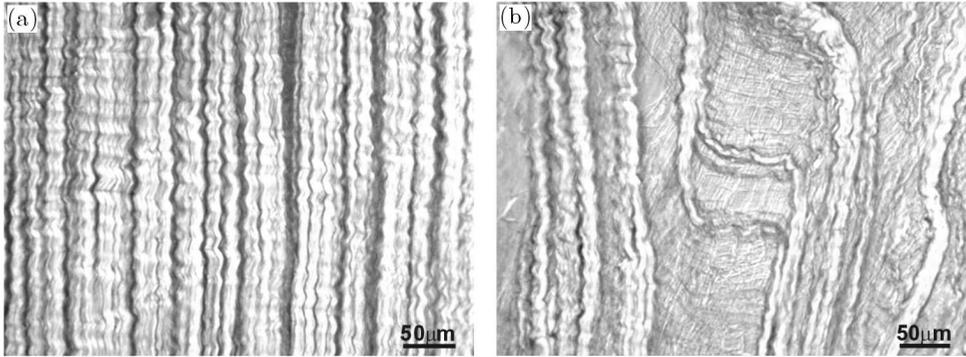


Fig. 7. Photographs of a single annulus fibrosus lamella: (a) in the unloaded state, (b) at the final transverse stretching stage

#### 4. Discussion

Research on single annulus fibrosus lamellae with preserved bone attachments have demonstrated two mechanisms of failure during stretching in the direction of the collagen fibres alignment.

In the first mechanism, the connection was broken between collagen fibres and the place of their anchoring, while in the second mechanism the collagen matrix was split and single collagen fibres split in the lamella area leading to slow lamella damage, and ultimately decreased the ability to transfer loads. Thanks to the large number of collagen fibres, the destruction process runs "smoothly", without sudden drops in force during the transfer of loads, as was described by Wagner and Lotz (2004). Additionally, permanent structural changes appearing during stretching prevent unequivocal determination of the changes in strain at the maximum tension.

It should be noted that the connection between borderline cartilage and bone is relatively weak, which makes this region vulnerable to damage. The strong interfibre structure (Broom, 1986) withstood the increasing stress, and damage often appeared at the bone-cartilage covering the bone surface.

Observation of the early stage of damage during stretching in two opposite directions in a microscopic setup may help to explain the fundamental issue: is high resistance to the stretching of annulus fibrosus due to the presence of long, unbroken collagen fibres running bone-to-bone (or cartilage-to-cartilage) or due to cohesion between short fibres, which despite lack of bone-to-bone fibres achieve a sufficient degree of consolidation to obtain high strength?

The second mechanism is proposed by numerous authors analysing the intervertebral disc strength, who quote the classic theory of composites with short fibres – Fig. 8 (Hukins *et al.*, 1985; Adams and Green, 1993; Green *et al.*, 1993).

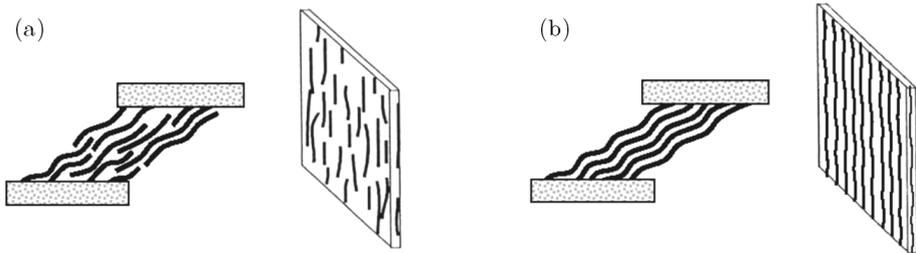


Fig. 8. A diagram showing the existence of: (a) short and (b) long collagen fibres constituting the structure of annulus fibrosus lamellae

During the stretching of micro-samples along collagen fibres, the maximum value of stress coincided with sudden commencement of the process of displacement and separation of fibres along their entire length as well as rapidly declining stress. This would suggest the first type of the damage mechanism, connected with anchoring of fibres in discs along their entire length, rather than just disintegration of cohesion (connection) between short, broken collagen fibres. This does not mean that there is no cohesion between the fibres. Rather, their role in contributing to high strength and stiffness of single annulus fibrosus lamellae is minor. This interpretation is further confirmed by the low levels of stress required to achieve progressive separation of subsequent collagen fibres once the largest scale of high value fibre separations has occurred.

The results obtained during stretching in the transverse direction to the alignment of collagen fibres of micro-samples also support and confirm the description of the strength of annulus fibrosus due to presence of long fibres anchored in the discs. The low, practically constant levels of stress values are required for the initial stretching of the parallel fibres followed by their reorientation, which is inconsistent with the model describing the strength and stiffness of annulus fibrosus resulting from a relatively strong cohesion between the fibres. What is important is that in such tissue structures there is a need for large flexibility of the structure itself with the simultaneous high strength and stiffness.

If the strength of annulus fibrosus comes from interconnection of short collagen fibres in the form of an intermediary structure, such as e.g. proteoglycans as proposed by Adams and Green (1993), then the structure should

be assigned as an important part of the obtained strength as is held by the fibres themselves. The problem with the theory presented this way is that if we do not deliver the appropriately high strength to the structure interconnecting short collagen fibres, then we will not obtain the appropriate link necessary to load from one fibre to the subsequent ones, which means the fibres will not be able to displace in relation to each other. A consequence of such an action, i.e. a very low level of fibre-structure interaction, is the obtainment of configuration of high elasticity, but with very low strength properties. At the same time, in such systems a growth in the strength of interconnecting (mediating) short collagen fibres may facilitate the transfer of loads transverse to the broken (non-uniform) collagen matrix, but at a cost of flexibility of that structure.

The problem looks differently in the load transferring the structure proposed by the author, i.e. a system of long, unbroken collagen fibres running from bone to bone. Such an arrangement provides appropriately all required properties, including: high flexibility, rapid stiffening as the crimp straightens reversibly, high rupture strength due to secure anchorage of the collagen fibres in the vertebral bone (or articular cartilage), and high toughness due to the large amount of mechanical work (or energy) needed to stretch the anchored fibres (Pezowicz, 2008).

Further evidence that the strength of the matrix is primarily due to the end-anchorage of the fibres rather than derived from significant fibre/matrix interactions is seen from the ease with which the collagen crimp is reversibly straightened. Additionally, the high level of interactions observed during stretching along and across the arrangement of collagen fibres could provide an effective protection of the fibre structure against simultaneous uncrimping in the whole matrix of annulus fibrosus.

#### **4.1. Model of annulus fibrosus as a layered orthopaedic structure**

Assuming, on the basis of conducted experimental investigations that subsequent annulus fibrosus lamellae are made of long collagen fibres, such a structure can be compared to fibrous multi-layer composites with differently oriented orthotropic layers.

In order to specify the stiffness characteristics of a single annulus fibrosus lamella, it was assumed that it would be considered as an orthopaedic layer, in the plane of which there is a plane stress state. The state is given by the components  $\sigma_1, \sigma_2, \sigma_6$ , ( $\sigma_3 = \sigma_4 = \sigma_5 = 0$ ), whose directions coincide with the main axes of orthotropic layers 1 and 2 (axis 1 – along fibres, axis 2 – perpendicular to the fibres in the layer plane).

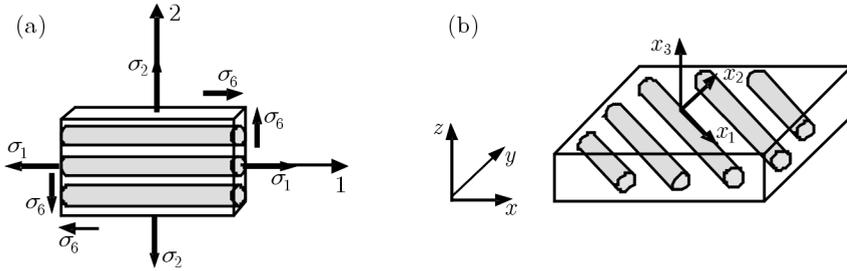


Fig. 9. The diagram shows a single annulus fibrous lamella in form of a unidirectional orthotropic layer: (a) unidirectional layer loaded along the main axes 1, 2; (b) global and material reference systems

In the analysed system, there exist two coordinate systems. The global coordinate system  $xyz$  refers to the whole composite body. Each layer is referred by own local coordinate system, in which the main axes are parallel to the axes specific to material –  $x_1x_2x_3$ . The axis  $x_3$  of each local coordinate system is parallel to the  $z$  axis of the global coordinate system. For a single layer consisting of collagen fibers parallel to  $x_1$ , the stress tensor consists of 4 independent components in the  $x_1x_2$  plane:  $C_{11}$ ,  $C_{22}$ ,  $C_{12}$ ,  $C_{66}$ .

The constitutive relation for a single-directional orthotropic layer number  $f$  can be described by Boczkowska *et al.* (2003)

$$\sigma^{(f)} = \mathbf{C}^{(f)} \varepsilon^{(f)} \tag{4.1}$$

$$\begin{bmatrix} \sigma_1 \\ \sigma_2 \\ \sigma_3 \end{bmatrix}^{(f)} = \begin{bmatrix} C_{11} & C_{12} & 0 \\ C_{21} & C_{22} & 0 \\ 0 & 0 & C_{66} \end{bmatrix}^{(f)} \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \varepsilon_3 \end{bmatrix}^{(f)}$$

Assuming mechanical properties of a single annulus fibrosus lamella, determined in the tensile test for micro-samples and the literature data collected in Table 2, we can determine the components of stiffness matrix using elements describing the stress and strain state of composites with unidirectional continuous fibres, by making use of the law of mixtures.

On the basis of the adopted mechanical properties, the stiffness matrix is obtained

$$\mathbf{C} = \begin{bmatrix} 331.2 & 397.5 & 0 \\ 26.3 & 37.5 & 0 \\ 0 & 0 & 0.1 \end{bmatrix}$$

The analysis of the structure of annulus fibrosus of the intervertebral disc clearly indicates that the direction of alignment of collagen fibres in a single la-

**Table 2.** Mechanical properties adopted to construct the stiffness matrix of a single annulus fibrosus lamella

Mechanical properties		Average value (SD)	Source
$E_{11}$ [MPa]	53	53.2 (27.5)	(Pezowicz <i>et al.</i> , 2005)
$E_{22}$ [MPa]	6	5.89 (3.12)	(Pezowicz <i>et al.</i> , 2005)
$\nu_{21}$	0.7	0.66 (0.22)	(Elliott and Setton, 2001)
$\nu_{12}$	1.2	1.16 (0.68)	(Elliott and Setton, 2001; Acaroglu <i>et al.</i> , 1995)
$G_{66}$ [MPa]	0.1	0.11 (0.03)	(Fujita <i>et al.</i> , 1996; Iatridas <i>et al.</i> , 1996)

mella corresponds to the model of a composite with long fibres and consecutive layers arranged at certain angle with respect to the stress direction  $\sigma_1$ .

The presented analogies between the annulus fibrosus and layered composite, strongly argue in favour of long fibres, which, by creating a system of consecutive fused lamellae, provide high mechanical resistance.

However, the described model is a large simplification of the actual construction and work of the annulus fibrosus of the intervertebral disc. Subsequent annulus lamellae are arranged at some angle to the disc axis, and additionally, the slope of collagen fibres at subsequent lamellae changes. The tilting slope of fibres at subsequent lamellae changes from the outside to the inside part of the disc (in the radial direction, Cassidy *et al.*, 1989). The collagen fibres of external lamellae are tilted at an angle of  $\sim 30^\circ$ , while in internal lamellae that angle increases to  $\sim 45^\circ$  – Fig. 10.

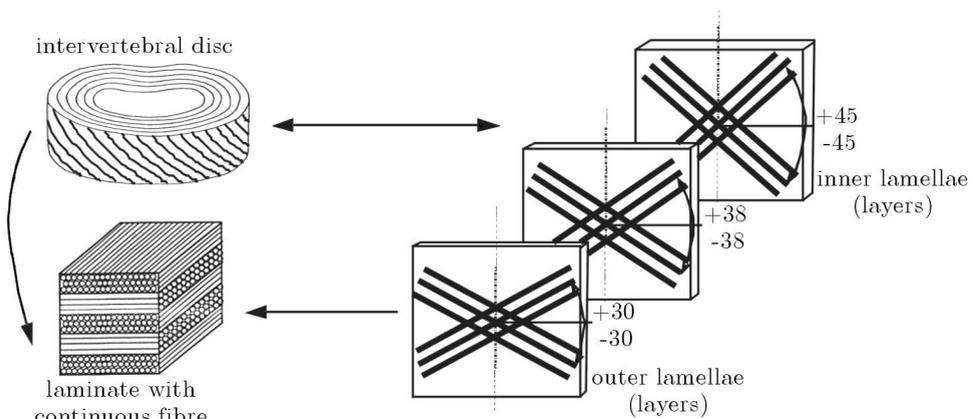


Fig. 10. A diagram showing the change in collagen fibre angle in subsequent annulus fibrosus lamellae

What is more, the internal lamellae differ in terms of construction compared to the external lamellae. Internal annuli are thicker (Marchand and Ahmed, 1990; Cassidy and Hiltner, 1989) and have a lower density of collagen fibres, i.e. they have a looser connection structure; additionally, they constitute a kind of transitional structure between the nucleus and the annulus because they contain the material of nucleus pulposus, which fills the spaces between the matrix of collagen fibres (Pezowicz *et al.*, 2006) – Fig. 10.

The presented results show that the process of degradation of the orthotropic structure of annulus fibrosus is closely dependent on the ongoing action of loading applied to the annular wall, which results in a slow process of dilution of the packing structure of collagen fibres and, consequently, to its irreversible damage. At the same time, depending on the loading direction in relation to the arrangement of collagen fibres, the characteristics of the strain process and the resultant degradation at a later stage are strongly related to the rate of strain and the internal cohesions of the annulus structure itself.

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### **Analiza właściwości mechanicznych krążka międzykręgowego kręgosłupa w skali makro i mikroskopowej**

#### Streszczenie

Celem pracy była doświadczalna analiza wybranych właściwości mechanicznych oraz zobrazowanie na poziomie makro i mikroskopowym, strukturalnej odpowiedzi na wymuszoną deformację w obszarze pojedynczej blaszki pierścienia, jak i zespołu kolejnych, połączonych ze sobą blaszek pierścienia włóknistego. Badania pojedynczej blaszki pierścienia z zachowanymi naturalnymi przyczepami do tkanki kostnej trzonów kręgow, wykazała istnienie dwóch mechanizmów zniszczenia tkanki oraz duże zróżnicowanie umownego modułu Younga w obszarach krzywej naprężeniowo-odkształceniowej. Badania na poziomie mikroskopowym umożliwiły zobrazowanie zmiany macierzy kolagenowej w trakcie rozciągania i istotnych zmian właściwości mechanicznych w zależności od kierunku obciążania.

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