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Spatial and structural dependence of mechanical properties of porcine intervertebral disc

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Structure–function relationship of natural tissues is crucial to design a device mimicking the structures present in human body. For this purpose, to provide guidelines to design an intervertebral disc (IVD) substitute, in this study the influence of the spatial location and structural components on the mechanical properties of porcine IVD was investigated.

Local compressive stiffness (LCS) was measured on the overall disc, also constrained between the two adjacent vertebrae: the dependence on the lumbar position was evaluated. The compliance values in the anterior position (A) were higher than both in the central posterior (CP) and in the lateral-posterior (RP, LP) locations. The values of Young's Modulus (74.67 ± 6.03 MPa) and compression break load ($1.36 \times 10^4 \pm 0.09 \times 10^4$N) of the disc were also evaluated by distributed compression test.

The NP rheological behavior was typical of weak-gels, with elastic modulus G' always higher than viscous modulus G'' all over the frequency range investigated (G' and G'' respectively equal to 320 and 85 Pa at 1 Hz) and with the moduli trends were almost parallel to each other.

Introduction

Today there is a compelling need for devices that are able to augment or substitute part of the human body. These devices have to reproduce biological and biomechanical functions of human tissues. It is necessary to understand the specific structure–function relationship, the structural and physical–chemical properties of biological materials to allow a new synthetic system to fulfil the same functions. The natural tissues, even if organized in different hierarchical manners, with a large variety of composition and structures, ultimately are composite materials, showing an anisotropic and viscoelastic behavior.

The intervertebral disc (IVD) is a complex structure that transmits and distributes large loads on the spine while providing flexibility [1]. The IVD is composed of three anatomical elements: the annulus fibrosis (AF), the nucleus pulposus (NP) and endplates (EP). AF consists of a fibrous collagen matrix embedded in a gel of proteoglycans. Structurally, collagens fibrils are oriented parallel to each other within lamellae [5]. NP is a relatively homogenous and highly gelatinous material, with a large amount of water (70–90% wet weight), proteoglycans (25–50% dry weight) and a network of collagen (10–20% dry weight) [2, 3]. The EP are cartilaginous material connecting IVD with vertebral bodies [12].

Spine deformation is allowed in three planes: flexion-extension, axial rotation and lateral bending [4]. However, bulging of discs during compressive loading indicates that axial load is transmitted by NP into radial and tensile stresses in the AF lamellae [6]. Moreover, it is believed that the mechanical role of NP is to provide for internal fluid pressurization in response to compressive or eccentric load related to the relative motion of vertebrae segments. The load support and shock load absorber function of IVD have been ascribed to these mechanisms [1, 7]. The goal of the current study was to investigate the spatial distribution of mechanical properties of IVD to provide guidelines for an IVD substitute design. The mechanical behavior of the IVD and its dependence on location were evaluated by compressive and shear tests [8].

Material and methods

A complete spinal column has been explanted from a pig (14 months old and weighing 150 kg) within 6 h of death. The discs in the lumbar zone (from level L2 up to L7) have been isolated by sawing horizontally adjacent vertebrae in their middle. These discs have been freed from ligament bounds, transverse and spinous process. The posterior articulating facets and pedicles were removed. The IVD system was thus constituted by an IVD constrained only between the two adjacent vertebrae half cut. Discs were enclosed in a plastic envelopment until tests, to avoid water loss. The samples were used fresh or frozen at 4 °C. In the last case, specimens were equilibrated at room temperature before tests. A total of

TABLE I Overall dimensions of three isolated segments from the lumbar region of the spine

Specimen	H_{tot} (mm)	T_{disc} (mm)	$2L$ (mm)	$2W$ (mm)
L2–L3	33	8	40	24
L3–L4	34	8	40	26
L4–L5	34	7	41	26
Mean	33.7	7.7	40.3	25.4
St. Dev.	0.58	0.58	0.58	1.15

six samples of IVD (L2–L3, L3–L4, L4–L5, L5–L6 and L6–L7) were tested.

Among the IVDs, three (from L2–L3 to L4–L5) were used to investigate the mechanical behavior of total disc according to possible spine movements, whereas two (L5–L6 and L6–L7) were employed to analyze the rheological properties of nucleus pulposus.

Two different kind of test were performed on the disc:

a. a local compression stiffness (LCS);
b. a distributed compression.

In LCS tests, four zones on the transversal plane of each IVD system specimens were considered: three posteriors (left posterior, central posterior and right posterior) and one anterior position (Fig. 1).

These zones were loaded in compression up to 50 N in order to investigate the dependence of the compliance as a function of location. The tests were carried out by using a MTS Bionix 858 Test System (MTS System Corporation, Minneapolis, MN 55424, USA) 25 kN load cell controlled in displacement at 1 mm/min, while the displacement was detected by an extensometer MTS model 632.32F-02.

After LCS tests, the distributed compression tests were performed on the same specimens as long as previous tests were performed within reversible elastic range of the material. The distributed compression tests were carried out until the breaking of the specimens was reached.

The total disc height was measured at the periphery. The cross-sectional area was valued by measuring the maximum width of the disc and the length along the saggittal plane. Then area formula for ellipse was applied adding 10% to the calculated value ($1.1\ \pi\ W\ L$)[10]. Strain values in stress–strain curve were measured by the MTS extensometer placed in anterior position.

Average and S.D. of the total disc height (H_{tot}), length ($2L$), width ($2W$) of specimens and these discs thickness (T_{disc}) are reported in Table I. The other IVDs (L5–L6 and L6–L7) were harvested and from these NPs were isolated. The mean of NPs weight was 110 mg.

Small amplitude oscillatory shear tests were performed by a strain-controlled rheometer Bohlin VOR (Bohlin Reologi AB, Lund, Sweden) at the controlled temperature of 37 °C.The samples were put in the humidity chamber of the testing apparatus to avoid the water loss. A plate–plate testing geometry (PP-15) was used. In particular, the dynamic elastic (storage) modulus $G'(\omega)$ and the viscous (loss) one $G''(\omega)$ were evaluated. G' gives an information about the energy stored in material during the deformation, while G'' measures the energy dissipated as heat.

Preliminary strain sweep tests were performed to determine the range of strain amplitude in which the material shows linear viscoelastic behavior. Water loss of NP before and after the tests was less than 5%.

Results and discussion

Generalized compression tests showed that the value of Young's Modulus for L2–L3 was 81 MPa while for L3–L4 and L4–L5 were respectively 74 and 69 MPa (Fig. 2).

The values of Young's Modulus (74.67 ± 6.03 MPa) and load at break in compression break load ($1.36 \times 10^4 \pm 0.09 \times 10^4$ N) of each specimen was also evaluated by distributed compression test.

The tests were stopped when a brittle rupture of samples was reached. In the uniaxial compression tests, the load at break ranged from 1.27×10^4 N (L2–L3) to 1.46×10^4 N (L4–L5) (Fig. 3). Each specimen displayed almost the same value of displacement at rupture (about 2.6 mm).

L2–L3 disc showed a higher Young's Modulus value with lower load (but intermediate stress) level at rupture. On the contrary, L4–L5 showed a lower Young's Modulus value but higher values of both load and stress at rupture (Figs. 2 and 3, Table II).

On the other hand, the LCS tests provided the values of

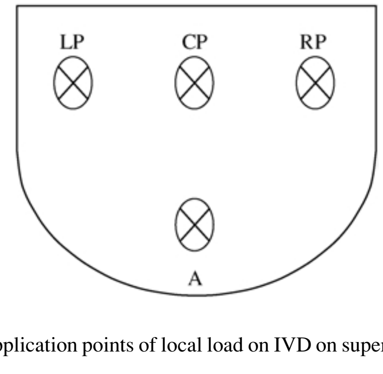

Figure 1 Application points of local load on IVD on superior surface of sample.

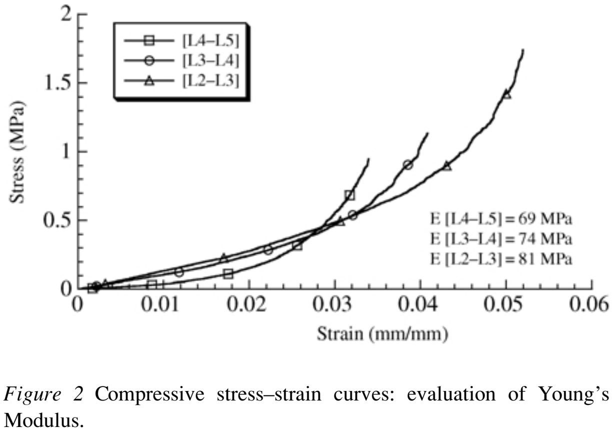

Figure 2 Compressive stress–strain curves: evaluation of Young's Modulus.

T A B L E I I Surface area along sigittal plane and mechanical properties of IVD tested

	L2–L3	L3–L4	L4–L5	Mean	St. dev.
Area (mm^2)	829	898	920	882.33	47.48
Young's Modulus (MPa)	81	74	69	74.67	6.03
Load at rupture (10^4 N)	1.27	1.35	1.46	1.36	0.09
Stress at rupture (MPa)	15.38	15.06	15.91	15.44	0.43

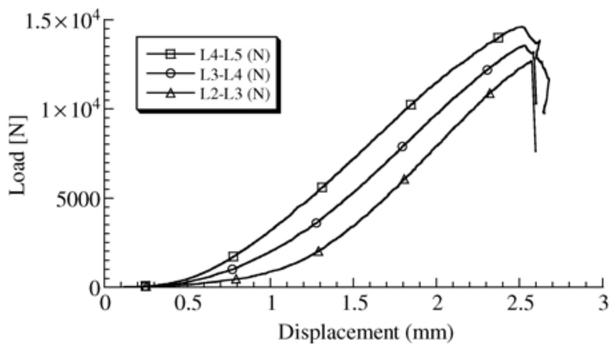

Figure 3 Compressive load-displacement curves: evaluation of load at rupture.

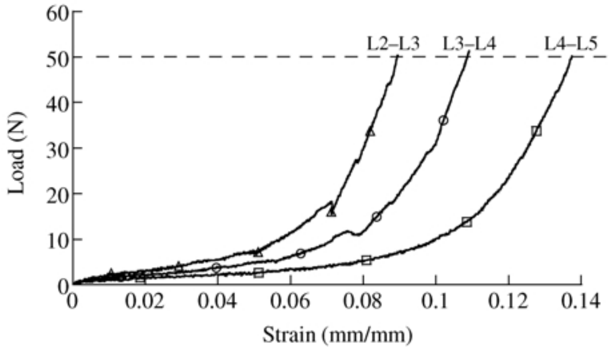

Figure 4 LCS: evaluation of compliance in anterior (A) zone.

the disc strain evaluated for a local load of 50 N (Table III), applied on four zones represented in Fig. 1. Moreover, Figs. 4–7 show the compliance curve up to 50 N.

Results showed a LCS variability of discs depending on the position on which the load is applied and on the lumbar region. Higher strain values occurred in anterior position (A) with lower compliance in L2–L3 disc and higher compliance was achieved in L4–L5, thus, in anterior position (A) the compliance increases in the L5 to L3 direction along the spine (Figs. 4–7)

By comparing anterior (A) and posterior compliances (CP, RP and LP), posterior ones show the smallest values. Indeed, the large value of strain obtained in anterior region (A) at 50 N was about twice than posterior ones. (Table III). In such case, L4–L5 disc showed the high slope and stiffness whereas L2–L3 disc presented a large amount of deformation at the same load value. As results, an increasing stiffness was suggested from lower lumbar disc (L4–L5) to upper one (L2–L3).

Lower mean values of compliance in the anterior region, compared with posterior ones, may be ascribable to wider possibility of antero movements of the column. This should be derived from the biomechanical functionality of the discs in the spine. The role of the disc is to provide a differentiated flexibility according to the deformation of the spine. In the same way, we might hypothesize that restricted posterior (CP) and posterior–

T A B L E I I I LCS of porcine IVD at 50 N

Position	Strain (mm/mm)	
	Mean	St. dev.
A	0.112	0.024
LP	0.039	0.018
CP	0.028	0.006
RP	0.036	0.009

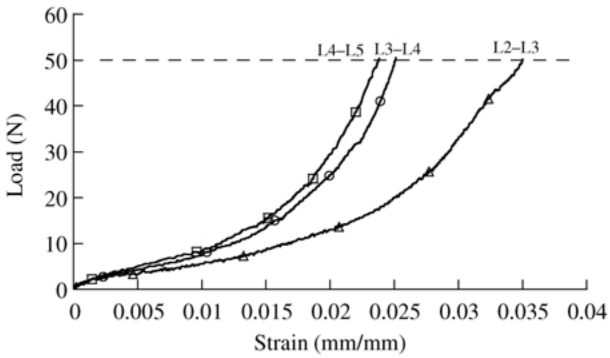

Figure 5 LCS: evaluation of compliance in central posterior (CP) zone.

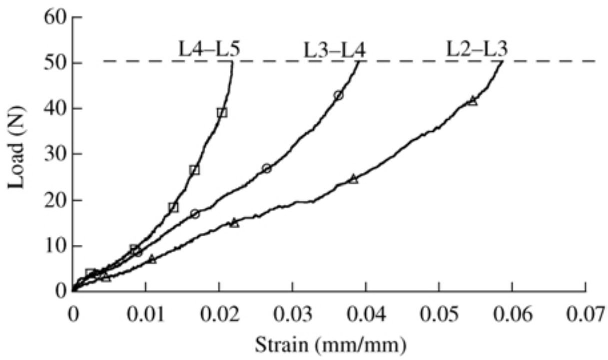

Figure 6 LCS: evaluation of compliance in left posterior (LP) zone.

lateral (RP, LP) movements should be consistent with the lower strain values obtained.

These findings may be explained by the anisotropic nature of IVD. Indeed, concentric fibril lamellae and proteoglycans matrix, making up IVD annulus, should differ morphologically from posterior–lateral to anterior region, varying from the morphological and compositional point of view.

To evaluate the viscoelastic properties of NP, small amplitude shear experiments have been performed in the frequency range of 0.01–10 Hz. Difference in weight before and after the test was evaluated less than 5%. The mechanical spectrum of NP is reported in Fig. 8. The NP exhibited a solid like behavior since G′ is always higher than G″ throughout the frequency range investigated, with both moduli trends almost parallel to each other [11]. Moreover, both G′ and G″ do not vary substantially as function of frequency. Also tanδ varies in a restricted range between 0.24 and 0.40. This rheological behavior is typical of a weak-gel.

Nucleus pulpous is a natural composite material made up of a viscoelastic matrix of highly hydrated GAG macromolecules reinforced with insoluble collagen fibrils. The viscoelastic matrix of nucleus pulpous is mainly composed by keratan-sulfate and choindroitin 4- or 6-sulfate, two kinds of glycosaminoglycans (GAG) [13]. These macromolecules behave in aqueous medium as expanded random coil because of the presence of SO_3^- and COO^- group on the chains; they occupy a large hydrodynamic volume overlapping with the other GAG macromolecules. It probably occurs through physical and topological interactions such as polymer ‘‘entanglements’’ and electrostatic bounds, responsible of the network structure. In nucleus, keratan and choindroitin sulfate may account for water retaining properties and provide a stabilizing effect on collagen structure as well as it occurs in other natural tissues [13].

The weak-gel behavior of the NP is related to its biological structure. The NP indeed plays a crucial role in biomechanics of the spine, contributing to maintaining the height of the disc under compressive loading and acting as a shock absorber for neighboring tissue thanks to its particular viscoelastic properties [9].

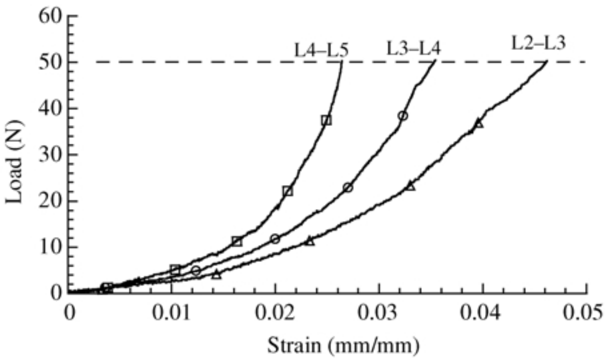

Figure 7 LCS: evaluation of compliance in right posterior (RP) zone.

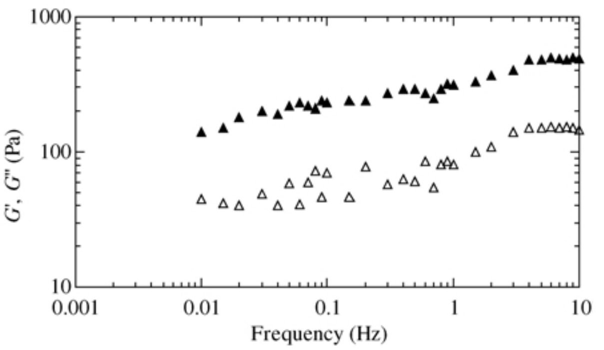

Figure 8 Mechanical spectrum of porcine NP at 37 °C.

Conclusion

Overall IVD properties were analyzed thoroughly by static point of view. The mechanical response of porcine IVD was also related to different local stiffness.

Moreover, dynamic mechanical analysis of NP was carried out. From these observations, IVD appears composed of a highly hydrated core, having a weak-gel rheological behavior, and external stiff tissue whose mechanical properties change locally.

Acknowledgments

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