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ABSTRACT

Experimental uniaxial tensile tests are carried out on circumferential samples removed from annulus tissue of pig intervertebral disks. Classical non-linear and hysteretic mechanical behaviors are observed. Using imaging techniques, the transverse dimensions of experimental samples are measured during stretching leading to the determination of both Poisson's coefficients. A large shrinkage is generally observed in the axial direction while a significant swelling is obtained in the radial direction. This emphasizes the complex coupling effects between the anisotropic fibrous structure and the viscous flow through the matrix.

This communication aims at presenting an anisotropic poro-elastic model able to describe the homogenized mechanical behavior (non-linearity, hysteresis) and to retrieve microstructural characteristics (fibers reorientation, coupling with the fluid phase). This characteristic should significantly influences the cell nutrients transport from vertebrae to the disk.

INTRODUCTION

The intervertebral disc is a highly-specialized element of the spine that provides flexibility and adsorbing capacities. When mechanical loads are transmitted along the spine, the intervertebral disc mainly supports compression and flexion stresses. This results in a hydrostatic excessive pressure in the nucleus pulposus and generates

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circumferential tensile stresses in the surrounding annulus fibrosus. Maximal physiologic strains observed along the annulus superficial tissue are about 4% when the disc is in compression or torsion, and about 6% when the disc is in flexion or extension [2], but never exceed 10% [6]. To hold these large circumferential stresses, the annulus tissue is composed of a woven oriented structure of collagen fibers inbuilt in a highly hydrated matrix. This structured layout provides some interesting mechanical properties, indeed, an anisotropic and non-linear behavior is observed when submitted to uniaxial tensile tests [8].

Using fiber-reinforced continuum theory, hyperelastic formulations have been developed to account for this non-linear elastic behavior. This leads to some exponential strain energy functions able to curve-fit the experimental stress-strain behaviors from various configurations: tension or compression on axial, radial or circumferential samples [5]. Nevertheless, with these homogenized approaches, the geometrical modifications of the micro-structure are hidden in the strain energy functions, what make tough to identify the micro-structural origin of the annulus fibrosus complex behavior.

In saturated porous media, the coupling effects between strains and fluid transfer are usually taken into account using a poro-elastic approach [1]. When submitted to uniaxial tension, the collagen fibers reorient toward the loading direction. Indeed, the fiber orientations could be the key parameter that govern the mechanical behavior of annulus in particular the fluid pressure. The goals of this communication is to develop a non-linear poro-elastic model able to describe the homogenized mechanical behavior (non-linearity, hysteresis) and to retrieve micro-structural characteristics (fibers reorientation, coupling with the fluid phase). This microstructural model is compared to experimental tests performed on pig annulus samples.

MATERIALS and METHOD

Specimen preparation

Lumbar discs (L1-L2) were harvested from cadaver of pig (160 Kg) obtained in a local slaughter house. To avoid a blood-clot formation inside the cartilage end plates, a subcutaneous injection of heparin (Innohep $4500UI$, tinzaparine, Leo Pharma) was administered the day before the sacrifice. Next, the spinal segment (L1-L5) was removed and stored into a 10% DMSO solution in a -86$°C$ freezer.

Later, the discs were separated from the vertebral bodies by blunt dissection and the posterior, lateral and anterior quadrants were separated from each disc using a surgical knife. From each quadrant (Fig. 1a), one or two plane-parallel specimens were extracted using a specific tool. This tool was realized with two razor blades separated with a 2 mm-spacer. Indeed, all specimens were excised in anatomical cut parallel to the horizontal plane along the circumferential direction $\vec{e}_\theta$ (Fig. 1a).

The length of each specimen was limited using a surgical knife. Then, to get stuck into the testing device grips, both specimen ends were glued into speckle aluminum rings using cyanoacrylate adhesive. Finally, 6 porcine specimens were prepared to analyze the unidimensional mechanical behavior along the circumferential direction

$\vec{e_\theta}$ and to measure the transversal thickness t_r and t_z respectively along the radial $\vec{e_r}$ and axial $\vec{e_z}$ direction.

Mechanical testing

Prior to testing, the specimens were stored in a $0.15\,Mol/l$ saline solution at $T = 37°C$. The testing device was composed of a Texture Analyzer (LF-Plus, Lloyd instrument) with a $50\,N$ load cell and two optical microscopes (ZEISS) equipped with digital video cameras (Fig. 1b). Each sample was placed into the testing device grips and stretched during 3 loading cycles from 0 to $1\,mm$ lengthening in order to reach a maximum strain of $\varepsilon_{max} = 10\%$. For all tests, the extension rate was $0.01\,mm/s$ and images were recorded every $2\,s$.

Numerical image analysis

Digital cameras provided 1024×768 pixels images leading to a spatial resolution of $1 \times 10^{-2}\,mm/pixel$. A digital image speckle correlation (DISC) method was used to measure the grip displacements allowing to compute the macroscopic specimen strain during stretching given by Eq. (1). This displacement field is used to generate a moving window applied on numerical images in order to track a material section of the specimen. Indeed, it is assumed that the mechanical behavior is macroscopically homogeneous and that cross-section remains perpendicular to the specimen axis. Then, using a thresholding technique, the lateral thicknesses t_r and t_z are evaluated at each time step (Fig. 1c).

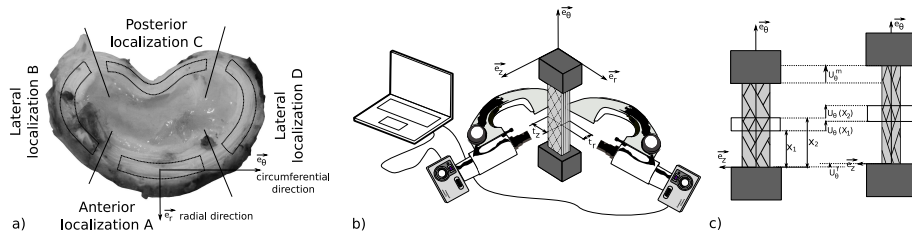$$U_\theta(x) = \frac{U_\theta^m(t) - U_\theta^f(t)}{l_0} x + U_\theta^f(t) \tag{1}$$

Figure 1 : a) Lumbar disc with the specimens locations; b) Specimen and optical microscopes used to evaluate the transversal dimensions. c) Moving window used to determine the transversal dimensions.

MICROSTRUCTURAL MODEL

The microstructure of annulus fibrosus is composed of collagen fibers included in the extracellular matrix. This matrix is mainly composed of water and elastin fibers which perform a junction between collagen fibers [9]. The collagen fibers govern the resistance to the annulus fibrosus while the matrix is responsible of its viscous behavior. The collagen fibers are oriented with an angle θ with respect to the vertical

plane. This angle θ generally ranges from $45°$ to $65°$ [3]. The annulus fibrosus is structured with concentric lamellae alternately oriented with an angle of $+\theta$ and $-\theta$.
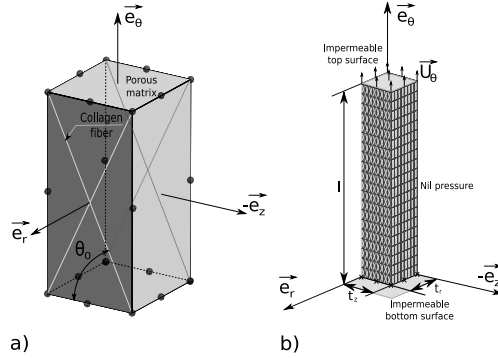

Figure 2 : a) A finite element of representative elementary volume (REV) of annulus fibrosus composed of oriented collagen fibers into porous matrix. b) Mesh of annulus fibrosus specimen.

The microstructure of an experimental sample (Fig. 2b) can be described by a regular layout of collagen fibers included in an homogeneous porous matrix (Fig. 2a). Collagen fibers are oriented with an initial angle θ_0 and represented by linear elastic cables characterized by an elastic modulus E_f. The elastic behavior is a fair assumption since they undergo low strains. The matrix, composed of an isotropic mixture of elastin fibers saturated with physiological liquid, is modeled through a poro-elastic behavior characterized by an elastic modulus E_m, a Poisson coefficient ν_m, a permeability k and a Biot coefficient b in drained conditions. Because, the solid and fluid fractions are nearly incompressible, this last parameter is fixed to $b = 1$. Thereby, the mechanical behavior of annulus fibrosus depends on 5 parameters: 4 mechanical parameters (E_f, E_m, ν_m, k) and 1 geometrical parameter θ_0. This poro-elastic model should be able to simulate both non-linear and anisotropic mechanical behavior of an annulus fibrosus specimen. Based on this description, a finite element model has been developed using code Aster.

RESULTS

Numerical image analysis provide the specimen transversal thicknesses in axial direction t_z and radial direction t_r. Transversal strains are computed using Eqs. (2) and (3). They are given in Fig. (3) as functions of the longitudinal strain $\varepsilon_{\theta\theta}$.

$$\varepsilon_{\theta z} = \frac{t_z - t_z^0}{t_z^0} \qquad (2) \qquad \varepsilon_{\theta r} = \frac{t_r - t_r^0}{t_r^0} \qquad (3)$$

With all specimens, a strong shrinkage in the transverse z-direction is generally observed. The axial strain $\varepsilon_{\theta z}$ reaches about 10% when the circumferential strain $\varepsilon_{\theta\theta} = 10\%$ (Fig. 3). On the opposite, regarding to the transverse r-direction $\varepsilon_{\theta r}$, different behaviors have been observed and two typical patterns are presented here. With specimen A1 (Fig. 3a), low variations in the r-direction is measured with a
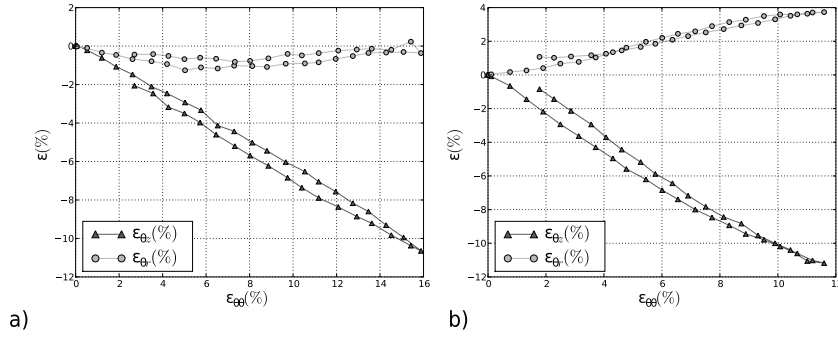
Figure 3 : Experimental curves of the transversal strains versus circumferential strain: a) Specimen A1, b) Specimen B2.

slight shrinkage at the beginning and a swelling at the end of stretching. Whereas, specimen B2 is characterized by a significant regular swelling up to $\varepsilon_{\theta r} = 4\%$.

Concerning the finite element model, the typical response of a poro-elastic structure is recalled in Fig. (4a) showing a shrinkage of the specimen in both transverse directions. The effect of collagen fibers, described by elastic cables, is clearly underlined in Fig. (4b). The transverse contraction $\varepsilon_{\theta z}$ is significantly increased when the collagen fibers reorient along the θ-direction. This leads to an additional pore pressure in the matrix that generates a bulge in the transverse r-direction.
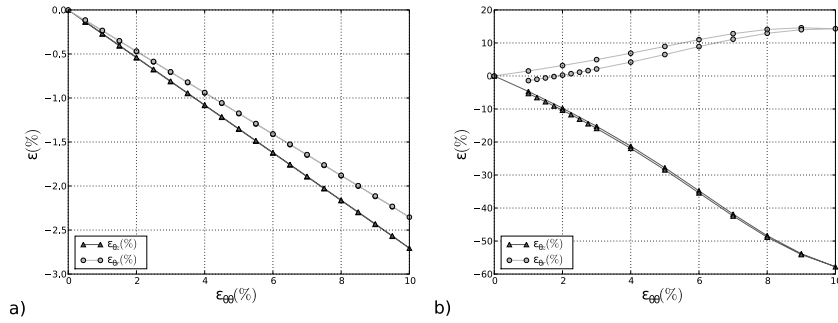

Figure 4 : Numerical results of the transverse strains versus circumferential strain computed: Finite element model (a) without fibers, or (b) with elastic fibers.

DISCUSSION

With a low number of parameters, 4 mechanical and 1 geometrical characteristics, this finite element model is able to represent several features of the mechanical behavior of annulus fibrosus tissue, i.e., non-linear stiffness, hysteretic visco-elastic behavior, reorientation of collagen fibers while loading and anisotropy in the $\vec{e}_\theta; \vec{e}_z$ plan.

It should be noted than, with this model, the strong non-linear behavior is obtained only for a limited range of initial fiber angle, $\theta_0 \approx 65°$. This initial angle is directly measured on outer annulus tissue, $\theta_0 \approx 65°$ [3]. With larger fibers orientation, as observed with inner annulus lamellae, the mechanical behavior is more linear [2, 8]. However, our tensile tests have been performed without preconditioning as usually done in previous experimental works [4, 2].

Considering the mechanical behavior of annulus fibrosus from a macroscopic point of view, two Poisson's coefficient can be determined, one associated with the r-direction, $\nu_{r\theta}$, and one with the z-direction, $\nu_{z\theta}$. In the lamellae plan, Poisson's coefficients measured are around $\nu_{z\theta} \approx 1$, which is largely above the limit associated with isotropic materials ($0 < \nu < 0.5$). Indeed, the micro-structure plays a major role since collagen fibers contract the porous matrix while they reorient along the loading direction. The shrinkage in the lamellae plan generates a positive pore pressure that makes the specimens swell in the direction perpendicular to the fibers. As observed with specimen B2, this swelling leads to negative macroscopic Poisson's coefficients $\nu_{r\theta} \approx -0.35$. This clearly shows that the macroscopic mechanical behavior highly depends on the liquid phase filtration through the porous matrix. These 3D coupling effects emphasizes the importance of flow characteristics when modeling the global disk behavior.

It must be noted that the strain computed with the finite element model are about ten times larger that the strain measured on experimental uniaxial tensile test. As investigated by Tower and *al.* [7], a transition occurs as the collagen fibers uncrimp becoming the primary load-bearing element. By imaging techniques, they show that a significant fiber reorientation takes place during the preconditioning and depends on the choice of the reference configuration. The fact that the fibers are initially crimped is not taken into account in our finite element model. Using non-linear cables generating no stress for low strain and an elastic response for higher strain should help when describing the mechanical behavior of annulus tissue. Anyway, even if this feature leads to a better representation of experimental test, in vivo, the annulus tissue is generally submitted to multiaxial stresses that keep the collagen fibers under tension.

REFERENCES

1. M. Argoubi and A. Shirazi-Adl. Poroelastic creep response analysis of a lumbar motion segment in compression. *J Biomech*, 29(10):1331–1339, Oct 1996.
2. E. C. Bass, F. A. Ashford, M. R. Segal, and J. C. Lotz. Biaxial testing of human annulus fibrosus and its implications for a constitutive formulation. *Ann Biomed Eng*, 32(9):1231–1242, Sep 2004.
3. J. J. Cassidy, A. Hiltner, and E. Baer. Hierarchical structure of the intervertebral disc. *Connect Tissue Res*, 23(1):75–88, 1989.
4. Y. Fujita, N. A. Duncan, and J. C. Lotz. Radial tensile properties of the lumbar annulus fibrosus are site and degeneration dependent. *J Orthop Res*, 15(6):814–819, Nov 1997.
5. S. M. Klisch and J. C. Lotz. Application of a fiber-reinforced continuum theory to multiple deformations of the annulus fibrosus. *J Biomech*, 32(10):1027–1036, Oct 1999.
6. A. Shirazi-Adl. Strain in fibers of a lumbar disc. analysis of the role of lifting in producing disc prolapse. *Spine*, 14(1):96–103, Jan 1989.
7. Theodore T Tower, Michael R Neidert, and Robert T Tranquillo. Fiber alignment imaging during mechanical testing of soft tissues. *Ann Biomed Eng*, 30(10):1221–1233, 2002.
8. Diane R Wagner and Jeffrey C Lotz. Theoretical model and experimental results for the nonlinear elastic behavior of human annulus fibrosus. *J Orthop Res*, 22(4):901–909, Jul 2004.
9. Jing Yu, Uday Tirlapur, Jeremy Fairbank, Penny Handford, Sally Roberts, C. Peter Winlove, Zhanfeng Cui, and Jill Urban. Microfibrils, elastin fibres and collagen fibres in the human intervertebral disc and bovine tail disc. *J Anat*, 210(4):460–471, Apr 2007.