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### Mechanical behaviour of annulus fibrosus: the role of the fluid phase

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#### 1. Introduction

The intervertebral disc is a highly-specialized element of the spine that provides flexibility and dissipative capacities. When mechanical loads are transmitted along the spine, the intervertebral disc mainly supports compression and bending stresses. This results in a hydrostatic excessive pressure in the central nucleus pulposus (NP) and generates circumferential tensile stresses in the surrounding annulus fibrosus (AF). To hold these large circumferential strains, the AF tissue is composed of a woven oriented structure of collagen fibres embedded in a highly hydrated matrix. This layout provides some interesting mechanical properties, i.e., an anisotropic and non-linear behaviour is observed when subjected to uniaxial tensile tests [3, 2, 1].

Actually, intervertebral disk tissues can be assimilated to porous media where the liquid phase flow plays a major role in the macroscopic mechanical behaviour. This is classically described by a poro-elastic formulation. The coupling effects between mechanical strains and viscous flows is of major importance when dealing with cell nutrition issues. Indeed, convective flows generated by macroscopic strains enhance to the nutrients transport from vertebrae towards NP cells. When subjected to loading cycles, alternating fluid flows can increase mass exchanges between inner and outer tissues. The strong anisotropy of AF properties could leads to particular flow repartitions that cannot be recovered by classical poro-elastic formulations.

#### 2. Materials and methods

Lumbar discs (L3-L4) were harvested from cadaver of a domestic pig and separated from the vertebral bodies by blunt dissection. From each quadrant (Fig. 1a), one plane-parallel  $(2 \times 2 \times 10 \text{ mm})$  specimen was carved out using a specific tool. Both specimen ends were glued into aluminium rings using cyanoacrylate adhesive (Fig. 1b).

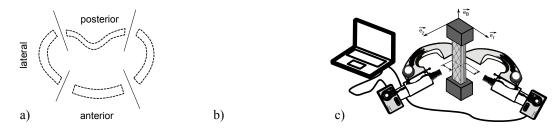


Figure 1: a) Lumbar disc with specimens locations; b) Annulus specimen with aluminium rings; c) Experimental device

The testing device was composed of a Texture Analyzer (LF-Plus, Lloyd Instruments) with a 50 N load cell. The fixed grip was placed inside a transparent bath filled with a 0.15 mol/l NaCl solution thermo-regulated at  $T = 37^{\circ}$ C. Two optical microscopes (ZEISS) equipped with digital video cameras were positioned perpendicularly to the tensile direction,  $\vec{e_{\theta}}$ , to visualise the transverse thicknesses of samples (Fig. 1c). Each sample was immersed into the thermo-regulated bath between the device grips and stretched during 3 loading cycles from 0 to 1 mm lengthening in order to reach a maximum longitudinal strain of  $\varepsilon_{max} = 10\%$ . Consecutively, a relaxation test was performed.

#### 3. Experimental results

Considering the evolution of the circumferential tensile stress as a function of strain, a strong nonlinear behaviour is systematically observed (Fig. 2a). This leads to the stiffening of the intervertebral disc tissue for large strains. Under loading cycles, a significant hysteresis is observed. This agrees with the essential dissipative function of intervertebral discs. In the second cycle, the dissipated energy is generally lower while the successive cycles converge towards a single curve.

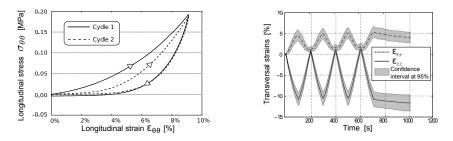


Figure 2: Annulus sample subjected to a cycling uniaxial tensile test - a) Longitudinal stress,  $\sigma_{\theta}$ , as a function of longitudinal strain,  $\varepsilon_{\theta\theta}$ ; b) Transverse strains,  $\varepsilon_{zz}$  and  $\varepsilon_{rr}$ , as functions of time, t.

Regarding to the strain in the plan of fibres,  $\varepsilon_{zz}$ , a linear reversible response is consistently observed (Fig. 2b). The Poisson's ratio computed are  $\nu_{\theta z} = 0.9 \pm 0.25$ . These values lie outside of the regular range valid for isotropic material,  $-1 \leq \nu \leq 0.5$ . Indeed, the transverse behaviour in the plan of fibres is governed by the reorientation of fibres along the loading direction. In the plan of lamellae,  $\varepsilon_{rr}$ , the linear swelling leads to a negative value of the Poisson's ratio. The strong transverse shrinkage in the plan of fibres generates a fluid over-pressure inside the porous matrix that discharges in the perpendicular direction, i.e., the direction of lamellae.

#### 4. Microstructural model

Based on these observations, a FEM of the AF tissue is proposed. It integrates lengthy elastic cables embedded in a poro-elastic matrix (Fig 3b). With few physical parameters, this model recovers the main features of annulus mechanical behaviour: non-linear stiffness, hysteresis, fibres reorientation, transverse strains (Fig. 3c). This model underlines the strong influence of the initial fibres angle, i.e., a non-linear behaviour is obtained only for a limited range of the initial fibres angle,  $\theta_0 \approx 25^\circ$ , close to the value directly measured on outer annulus.

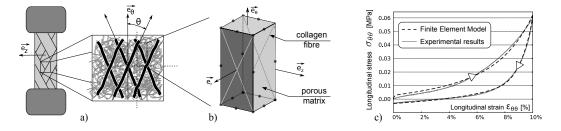


Figure 3: a) Description of internal microstructure of AF tissue; b) Finite element model of AF microstructure; c) Comparison between experimental measures and computed behaviour.

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