



**HAL**  
open science

# A microstructure-based modeling approach to assess aging-sensitive mechanics of human intervertebral disc

Karim Kandil, Fahmi Zaïri, Tanguy Messenger, Fahed Zaïri

## ► To cite this version:

Karim Kandil, Fahmi Zaïri, Tanguy Messenger, Fahed Zaïri. A microstructure-based modeling approach to assess aging-sensitive mechanics of human intervertebral disc. *Computer Methods and Programs in Biomedicine*, 2020, 200, pp.105890. 10.1016/j.cmpb.2020.105890 . hal-03635201

**HAL Id: hal-03635201**

**<https://hal.science/hal-03635201>**

Submitted on 10 Mar 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## A microstructure-based modeling approach to assess aging-sensitive mechanics of human intervertebral disc

Karim Kandil<sup>a,b</sup>, Fahmi Zaïri<sup>b\*</sup>, Tanguy Messenger<sup>a</sup>, Fahed Zaïri<sup>c</sup>

<sup>a</sup>Lille University, Unité de Mécanique de Lille (EA 7572 UML), 59000 Lille, France

<sup>b</sup>Lille University, Civil Engineering and geo-Environmental Laboratory (ULR 4515 LGCgE), 59000 Lille, France

<sup>c</sup>Ramsay Générale de Santé, Hôpital privé Le Bois, 59000 Lille, France

\*Corresponding author. [fahmi.zairi@polytech-lille.fr](mailto:fahmi.zairi@polytech-lille.fr)

### Abstract

**Background and objective:** The human body soft tissues are hierarchic structures interacting in a complex manner with the surrounding biochemical environment. The loss of soft tissues functionality with age leads to more vulnerability regarding external mechanical loadings and increases the risk of injuries. As a main example of the human body soft tissues, the intervertebral disc mechanical response evolution with age is explored. Although the age-dependence of the intervertebral disc microstructure is a well-known feature, no noticeable age effect on the disc stiffness is evidenced in the in-vitro experimental studies of the literature. So, if the disc intrinsic mechanics remains constant, how to explain the correlation of disc degeneration and disc functionality loss with age.

**Methods:** A microstructure-based modeling approach was developed to assess in-silico the aging-sensitive mechanics of human intervertebral disc. The modeling considers the relationship between stress/volumetric macro-response and microstructure along with effective age effects acting at the lamellar and multi-lamellar scales. The stress-stretch and transversal responses of the different disc regions were computed for various age groups (13-18, 36, 58, 69 and 82 years old) and their evolution with age was studied.

**Results:** While matching with in-vitro experimental data, the predicted stiffness was found to be increasing while passing from adolescent young discs to mature older discs then remaining almost constant for the rest of life. Important age-related changes in the disc transversal behavior were also predicted affecting the flexibility of the disc, changing its volumetric behavior, and modifying its dimensions.

**Conclusion:** The developed approach was found able to bring new conclusions about age-dependent mechanical properties including regional dependency. The disc mechanics in terms of rigidity, radial and axial transversal responses were found to alter going from adolescent to middle age where the disc reaches a certain maturity. After reaching maturity, the mechanical properties undergo very slight changes until becoming almost constant with age.

**Keywords:** Human annulus fibrosus; Aging; Mechanics; Regional dependency; Microstructure.

## **1. Introduction**

Intervertebral disc dysfunctions are the main factors of chronic/severe back pain affecting humans at any stage of life but staying highly correlated to age with excessive higher vulnerability of elders [1-2]. The damage of this living soft tissue could occur mechanically under excessive repetitive efforts, wrong movements and high loads supported by the spinal body [3]. Through recent years, big efforts were done in medicine to create patient-specific models of the different human body organs [4-6]. Nonetheless, if we need to have a realistic and accurate model of each patient, these models should refer to the actualized material properties of the organ and their evolution under the different degradation mechanisms principally caused by aging. Obviously, the mechanically-induced damage is influenced by intervertebral disc age-related changes. Natural aging induces, by essence, biological degeneration events [7] interacting with the intervertebral disc mechanics and creating complex degeneration processes inside the intervertebral disc lamellar fibrillar cartilage (annulus fibrosus) along with complex changes of interactions with the gelatinous central nucleus pulposus leading to disc morphological changes and sagittal balance disorders [8]. Micro-cracks and tears are common damage mechanisms observed in the annulus fibrosus [9-11]. Any alteration in the annulus fibrosus structure potentially leads to several forms of degeneration such as disc bulging, herniated disc and disc thinning. The comprehension of annulus microstructure and its relation to the natural aging is the key point in order to better understand the origin of these degeneration mechanisms, to reduce their effects and in some cases to avoid their occurrence. Distortions of the nutrient distribution and metabolism alteration appear inside the disc with age affecting directly the cellular density and indirectly the proteoglycan content, the volumetric pressure and the water content [12-17]. As well, age affects the total collagen fibers concentration and changes the proportions of the different types of collagen [18] which would logically alter the mechanical behavior of the annulus in

terms of rigidity and resistance. Many experimental studies tried to find a relation between the degeneration mechanisms and the microstructure changes of the disc components by natural aging [19] and their consequences on the disc mechanics [20-22]. Surprisingly, no effect of age was found on stiffness of single and multi-lamellar specimens of the annulus soft tissue [23-25]. In the latter references, it was deduced that the mechanics of a healthy disc remains constant at any stage of its life. These observations raise several questions about the structure-mechanics relationship in disc tissues, such as how the amount of the disc microstructure compositions may change without impact mechanical behavior and why elders are more likely to be touched by disc diseases compared to young individuals. Answering these questions is very difficult from the experimental viewpoint due to the huge number of specimens needed from cadavers of various ages and the extensive mechanical and biological studies that should be established on each specimen without damaging it. Computer modeling remains the best way for this kind of complex investigations. Many constitutive models tried to describe the mechanical behavior of the intervertebral disc going from the firstly developed models describing the annulus tissue as a single solid phase reinforced by stiff oriented fibers [26-29], arriving to biphasic and extended biphasic models where the fluid phase, its diffusion inward/outward the disc and in addition the negative charges density responsible of the water absorption inside the disc and the tissue swelling due to osmotic effect were considered [30-34]. All the recent models were able to reproduce the instantaneous disc response under external mechanical loads without a real connection with the disc microstructure and biology making them unable to estimate long-term temporal changes inside the disc. The strong bio-mechanical coupling is not considered in these models. The effect of temporal biological changes on the disc mechanics and the inverse effect of the disc mechanics on the biological behavior and the disc metabolism were not considered. Hence, metabolic bio-mechanical models appeared focusing on the cellular viability mechanisms, the nutrient diffusion

(oxygen, glucose and lactate) and the effect of applied mechanical loads on their distribution all over the disc [35-41]. Recently, these models evolved largely by incorporating the influence of the nutrient distribution and the disc metabolism on the cells synthesis, the microstructure remodeling of the proteoglycan network and the water content [42-44] and their incidence on the stress distribution inside the disc. Despite the important advance accomplished by these models and their promising results, they cannot yet investigate sufficiently the global long-term effects on the mechanical behavior of the disc tissues. None of these models highlight the inverse temporal effect of the microstructure changes on the mechanical properties of the soft tissue which could alter completely its response to the different mechanical loads and highly change the estimated results. For this reason, a recently developed microstructure-based computational model [45-47] that succeeded to reproduce the axial and multiaxial mechanical behavior of the disc annulus is used to assess the natural aging effects on the disc mechanics. Since the annulus Poisson's ratios (that is the negative of the ratio between the transversal strains and the applied circumferential strain) were found experimentally to be time-dependent [48-49], the model takes into account the transversal behavior of the annulus and its volumetric response that are mandatory to give a correct three dimensional description of the disc behavior [45-46]. In order to focus on the direct relation between the microstructure and the mechanical response, the metabolic origins of the microstructure changes [42, 50] were not considered in this research but the resulting altered microstructure contents of these components were taken directly from well-documented papers of the literature depending on the studied age and were adapted in order to be used by the model. The impact of age on the mechanical response in terms of stress-strain and transversal strains is investigated by varying for each disc region the oriented collagen fibers and the water contents along with the fibers orientations. The adopted approach allows us to study the age effect in a simple manner and reach almost the same final conducts. The study

was established on different annular and radial disc regions. The response of each region was investigated for disc models of several ages varying from young adolescents to elderly (13-19, 36, 58, 69 and 82 years old). Detailed analysis of the computational curves and the local stress-strain fields is performed. Important microstructure explanations about previously found experimental results are evoked and new conclusions about age-dependent mechanical properties are revealed.

This paper is organized as follows. Section 2 gives the details of the microstructure-based model presented at the scale of a single-lamellar annulus and at the scale of a multi-lamellar annulus. Section 3 presents and discusses our findings on the consequences of natural aging on the disc mechanics. Finally, some concluding remarks are given in Section 4.

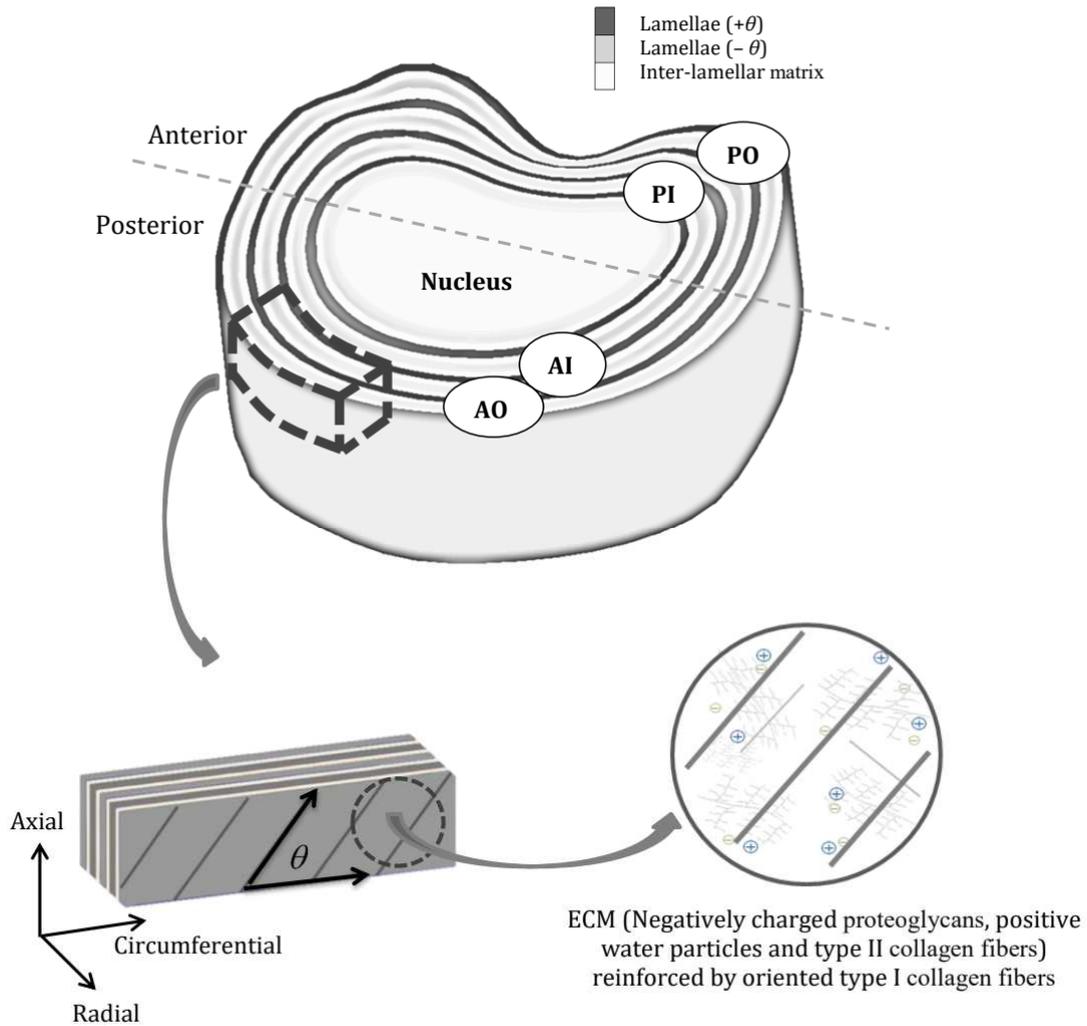


Figure 1. Multi-scale view of the disc tissues.

## 2. Microstructure-based model

Fig. 1 presents a multi-scale view of the disc tissues. Due to the heterogeneity of the disc microstructure [51-53], the disc annulus was divided circumferentially and radially into four regions: Posterior-Outer (PO), Posterior-Inner (PI), Anterior-Outer (AO) and Anterior-Inner (AI) that will be considered in the different calculations. The lamellae of annulus fibrosus are composed of ECM rich of negatively charged proteoglycans that are responsible of the disc hydration and containing non-oriented type II collagen fibers that are dispersed randomly all over the matrix. The lamellae are reinforced by oriented type I collagen fibers giving the soft tissue the required strength and helping the disc to resist the different applied external

mechanical loads. Each two adjacent lamellae are connected by a non-fibrillar ECM (inter-lamellar matrix) containing fibrils, elastin, crosslinks, and other bridging elements ensuring the structure integrity [54].

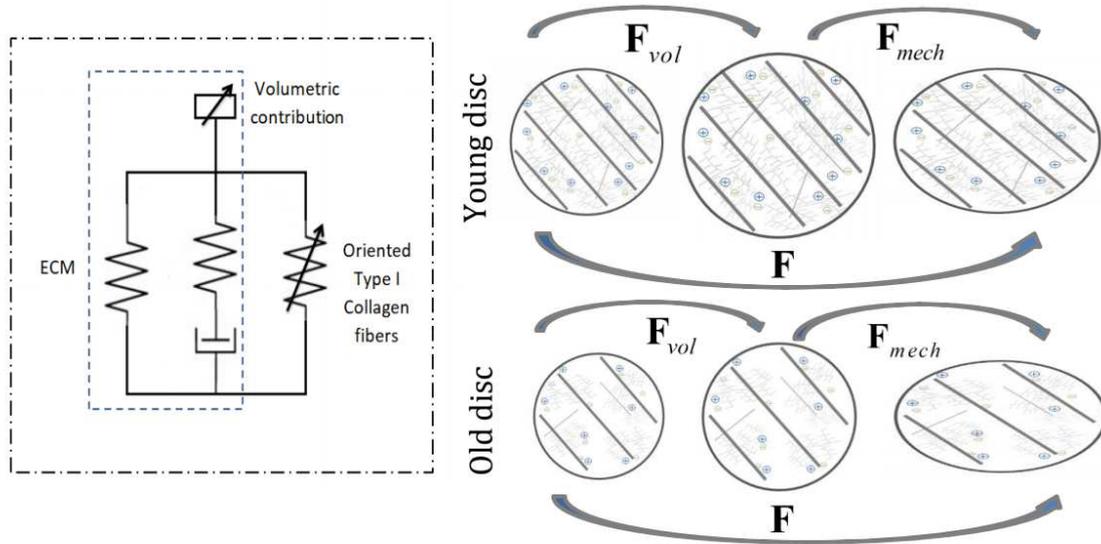


Figure 2. One-dimensional visualization of the model for single-lamellar annulus; the ECM deformation  $\mathbf{F}$  is split into a volumetric part  $\mathbf{F}_{vol}$  related to the chemical-induced expansion and a mechanical part  $\mathbf{F}_{mech}$  related to the intrinsic ECM response consisting in a nonlinear elastic spring in parallel with a Maxwell branch (a nonlinear elastic spring in series with a viscous damper). The elastic contribution of oriented type I collagen fibers acts as a nonlinear elastic spring in parallel.

## 2.1. Single-lamellar annulus model

The annulus behavior was reproduced using the recently developed microstructure-based chemo-viscoelastic model in [46-47]. The annulus fibrosus mechanical behavior is composed of the viscoelastic contribution of the ECM, the elastic contribution of oriented type I collagen fibers and the volumetric contribution of the ECM induced by the high density negative charges present in its proteoglycans network and responsible of the osmotic water transfer inside and outside the disc. Fig. 2 gives a rheological representation of the constitutive model along with the finite-strain kinematics. The free energy function  $\psi$  of the annulus fibrosus tissue is given by:

$$\psi = (1 - \phi_{CF})\psi_{ECM} + \phi_{CF}\psi_{CF} + (1 - \phi_{CF})\psi_{vol} \quad (1)$$

where  $\phi_{CF}$  is the volume fraction of oriented type I collagen fibers,  $\psi_{ECM}$ ,  $\psi_{CF}$  and  $\psi_{vol}$  are the free energy functions from which the stress-strain relationship along with the transversal behavior are obtained.

### 2.1.1. Free energy of ECM

The ECM free energy function  $\psi_{ECM}$  is split into an elastic part  $\psi_{ECM}^e$  and a viscous part  $\psi_{ECM}^v$  as:

$$\psi_{ECM} = \psi_{ECM}^e + \psi_{ECM}^v \quad (2)$$

The elastic free energy function  $\psi_{ECM}^e$  uses a Gent expression [55]:

$$\psi_{ECM}^e = -\frac{E}{6} I_1^{\max} \ln \left( 1 - \frac{I_1 - 3}{I_1^{\max}} \right) \quad (3)$$

where  $E$  is the ECM tensile modulus,  $I_1^{\max}$  is the ECM limiting extensibility and  $I_1 = \text{tr}(\mathbf{B}_{mech})$  is the first strain invariant with  $\mathbf{B}_{mech} = \mathbf{F}_{mech} \mathbf{F}_{mech}^T$  the mechanical left Cauchy-Green strain tensor.

The viscous free energy function  $\psi_{ECM}^v$  uses also a Gent expression [55]:

$$\psi_{ECM}^v = -\frac{E_v}{6} I_{1v}^{\max} \ln \left( 1 - \frac{I_{1e} - 3}{I_{1v}^{\max}} \right) \quad (4)$$

where  $E_v$  and  $I_{1v}^{\max}$  are ECM viscous constants and  $I_{1e} = \text{tr}(\mathbf{B}_e)$  is the first strain invariant with  $\mathbf{B}_e = \mathbf{F}_e \mathbf{F}_e^T$  the mechanical elastic left Cauchy-Green strain tensor and  $\mathbf{F}_e = \mathbf{F}_{mech} \mathbf{F}_v^{-1}$ . By introducing the convenient hypothesis of viscous irrotationality [56], the mechanical viscous deformation gradient tensor  $\mathbf{F}_v$  is driven by the viscous stretching rate tensor  $\mathbf{D}_v$ :

$$\dot{\mathbf{F}}_v = \mathbf{F}_e^{-1} \mathbf{D}_v \mathbf{F}_{mech} \quad (5)$$

with the following general flow rule:

$$\mathbf{D}_v = \dot{\gamma}_v \frac{\boldsymbol{\sigma}'_v}{\sqrt{2}\|\boldsymbol{\sigma}_v\|}, \|\boldsymbol{\sigma}_v\| = \sqrt{\frac{1}{2}\text{tr}(\boldsymbol{\sigma}'_v\boldsymbol{\sigma}'_v)}, \boldsymbol{\sigma}'_v = \boldsymbol{\sigma}_v - \frac{1}{3}\text{tr}(\boldsymbol{\sigma}_v)\mathbf{I} \quad (6)$$

in which  $\dot{\gamma}_v$  is the accumulated viscous strain rate,  $\|\boldsymbol{\sigma}_v\|$  is the effective value of the viscous Cauchy stress  $\boldsymbol{\sigma}_v$ ,  $\boldsymbol{\sigma}'_v$  is the deviatoric part of  $\boldsymbol{\sigma}_v$  and  $\mathbf{I}$  is the identity tensor.

The mathematical formulation of Bergstrom and Boyce [57] is retained for  $\dot{\gamma}_I^{\text{vp}}$ :

$$\dot{\gamma}_v = d \left| \sqrt{I_{1v}/3} - 1 \right|^{-m} \|\boldsymbol{\sigma}_v\| \quad (7)$$

where  $d$  and  $m$  are two supplementary ECM viscous constants and  $I_{1v} = \text{tr}(\mathbf{B}_v)$  is the first strain invariant with  $\mathbf{B}_v = \mathbf{F}_v\mathbf{F}_v^T$  the mechanical viscous left Cauchy-Green strain tensor.

### 2.1.2. Free energy of oriented type I collagen fibers

The free energy function  $\psi_{CF}$  of oriented type I collagen fibers is given by [58]:

$$\psi_{CF} = A_1(I_4 - 1) + A_2(I_4 - 1)^2 - 2A_1 \ln(\lambda_I^{x^2} \lambda_{II}^{y^2} \lambda_{III}^{z^2}) \quad (8)$$

where  $A_1$  and  $A_2$  are material constants,  $\lambda_I$ ,  $\lambda_{II}$  and  $\lambda_{III}$  are the stretches along the principal axes of oriented type I collagen fibers:

$$\lambda_I = \sqrt{\mathbf{e}_1 \mathbf{C}_{mech} \mathbf{e}_1}, \lambda_{II} = \sqrt{\mathbf{e}_2 \mathbf{C}_{mech} \mathbf{e}_2}, \lambda_{III} = \sqrt{\mathbf{e}_3 \mathbf{C}_{mech} \mathbf{e}_3} \quad (9)$$

with  $\mathbf{C}_{mech} = \mathbf{F}_{mech}^T \mathbf{F}_{mech}$  the mechanical right Cauchy-Green strain tensor. The term

$I_4 = \mathbf{a} \mathbf{C}_{mech} \mathbf{a}$  is the fourth strain invariant with  $\mathbf{a} = x\mathbf{e}_1 + y\mathbf{e}_2 + z\mathbf{e}_3$  the unit vector in the initial configuration as illustrated in Fig. 1.

### 2.1.3. Free energy of chemical-induced volumetric part

The chemical-induced volumetric free energy function  $\psi_{vol}$  is expressed as:

$$\psi_{vol} = \frac{1}{4}k(J^2 - 1 - 2\ln J) \quad (10)$$

where  $k$  is the bulk modulus and  $J = \det \mathbf{F}$  is the Jacobian given by:

$$J = J_{mech}J_{vol} = n_{f_m}\xi\eta, J_{mech} = \det \mathbf{F}_{mech} = 1, J_{vol} = \det \mathbf{F}_{vol} \quad (11)$$

in which  $J_{mech}$  and  $J_{vol}$  represent the intrinsic mechanical and chemical-induced volume changes, the chemical deformation gradient of the isotropic swelled continuum medium is  $\mathbf{F}_{vol} = J^{1/3}\mathbf{I}$ ,  $\xi$  is a dimensionless transportation coefficient,  $\eta$  is a dimensionless free swelling coefficient (equal to 0.5 in the case of physiological salt condition) and  $n_{f_m}$  is the internal fluid content governed by the kinetics:

$$\dot{n}_{f_m} = \beta_m \left( 1 - \frac{n_{f_m}}{n_{f_{lim}}} \right) \quad (12)$$

The osmotic water transfer inside and outside the annulus tissue is controlled by  $\Delta n_{f_m}$  where  $\beta_m$  is a fluid flow constant,  $n_{f_{lim}}$  is the maximum fluid content in the tissue and  $n_{f_0}$  is the fluid content at  $t = 0$  in the unloaded chemically equilibrated state.

## 2.2. Multi-lamellar annulus model

A representative volume of the annulus fibrosus specimen that takes into account the lamellar structure of the annulus and the presence of the inter-lamellar matrix, that was found recently to play a key role in the disc mechanics [45, 59-61], was designed following the same recommendations of Kandil et al. [46]. A uniaxial circumferential tension strain of 10% was applied to all specimens under a strain rate of 0.002 /s.

Parameter	Unit	Significance	Value
$E$	MPa	ECM stiffness	1.0
$I_1^{\max}$	MPa	ECM limiting extensibility constant	3.0
$E_v$	MPa	ECM viscosity	0.167

$I_{1v}^{\max}$	MPa	ECM viscosity	1.5
$d$	$\text{MPa}^{-1}\text{s}^{-1}$	ECM viscosity	0.02
$m$		ECM viscosity	0.001
$A_1$	MPa	Collagen stiffness	27.0
$A_2$	MPa	Collagen stiffness	100.0
$\beta_m$	$\text{s}^{-1}$	Fluid flow constant in lamella	-0.0004
$\beta_m$	$\text{s}^{-1}$	Fluid flow constant in inter-lamellar matrix	0.00097
$k$	MPa	Inner Bulk modulus	800
$k$	MPa	Outer Bulk modulus	3500
$\xi$		Inner transportation coefficient in lamella	40.25
$\xi$		Outer transportation coefficient in lamella	26.4
$\xi$		Inner transportation coefficient in inter-lamellar matrix	15.29
$\xi$		Outer transportation coefficient in inter-lamellar matrix	7.65

Table 1. Model constants.

The model succeeded to reproduce the stress/volumetric behavior of the bovine annulus fibrosus [46] and was extended later in [47] to simulate the behavior of the human annulus fibrosus by adjusting the fluid kinetics and the microstructure contents. The model parameters have been the outcome of an identification procedure that consisted to minimize the difference between a few 1D response dataset (stress-strain and transversal response) and model simulations using a hybrid trans-species experimental/modeling strategy [47]. Our modeling approach allowed to predict the 3D response dataset of the different human disc regions. More details of the identification procedure and model verification can be found elsewhere [47]. The model constants are listed in Table 1. Note that the fluid kinetics are provided here for the considered loading rate mentioned before.

The human model was implemented into the non-linear finite element software MSC.Marc using a set of subroutines constituted by (i) a hyperelastic subroutine to account for the ECM and fibers elasticity, (ii) a viscous subroutine to account for the ECM viscosity, and (iii) a volumetric subroutine to account for the fluid phase contribution. A flowchart of the model implementation procedure is represented in Fig. 3. The reader is also referred to

complementary references [62-63] for the general implementation procedure of coupled models.

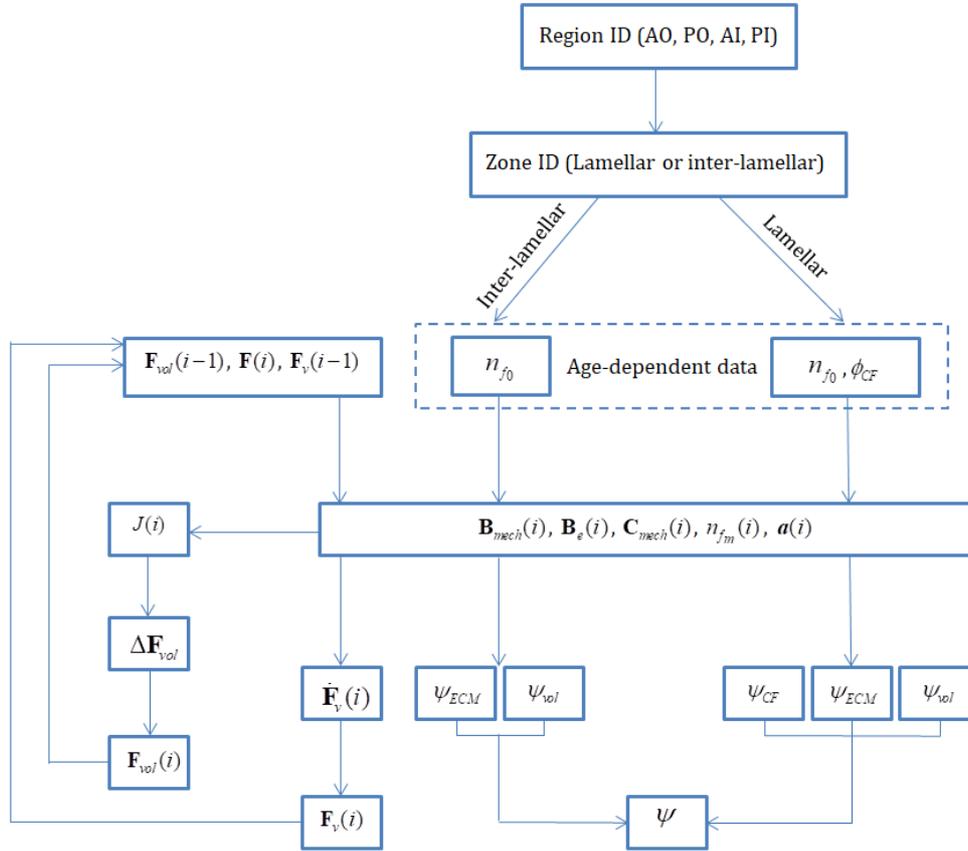


Figure 3. Flow chart of the model implementation. It considers the structural features of the studied region and the studied zone by mentioning their corresponding identification (ID) which then affects their corresponding microstructure quantities and free energy functions.

### 2.3. Aged-related microstructure changes

We tried to investigate the effect of the microstructure changes induced by natural aging on the mechanical behavior of the annulus tissue. For each disc region, the average water content and type I collagen fibers and orientation was assigned depending on the studied disc age. Five different disc ages (13-18, 36, 58, 69 and 82 years old) going from adolescents to elderly were considered. In order to calculate the wet density of type I collagen fibers  $\phi_{CF}$  the following equation was used:

$$\phi_{CF} = (1 - n_{unloaded}) \phi_{CF(I+II)} \phi_{CF(I/(I+II))} \quad (13)$$

where  $\phi_{CF(I+II)}$  is the average total collagen fibers and  $\phi_{CF(I/(I+II))}$  is the type I collagen fibers over the total collagen fibers. All fibers data were taken for L3-L4 disc. The values are provided in Table 2 for each region.

<b>Age</b>	<b>AO</b>	<b>AI</b>	<b>PO</b>	<b>PI</b>
Less than 18	66.5 (68.9)	54 (36.2)	66.5 (68.9)	53.33 (36.25)
36	65.4 (70)	52 (43)	68.65 (79.1)	54.55 (35.95)
58	64.25 (69.45)	50 (49.2)	70.75 (85.7)	56.15 (35.15)
69	62.45 (63.65)	48.9 (49.7)	72.15 (83.85)	57.8 (33.65)
82	60.5 (58.95)	48.1 (48)	76.65 (78.1)	60.45 (35.1)

Table 2. Total collagen and type I collagen contents in each disc region for different ages. Between brackets (type I collagen content over all collagen content). All values are extracted from [18] where the annulus width was divided into four parts. The collagen concentrations of the two outer parts were averaged for AO and PO and the concentrations of the two inner parts were averaged for AI and PI.

Fibers angle values were chosen to be 23° for AO, 30° for AI and 48° for PO and PI [25]. Since no available data was found about the initial water content in each disc region for the same ages, the average water content evolution was computed by a logarithmic regression of Koeller et al. [21] data and providing average water content change in lumbar disc with age. The calculated curve was adapted for each disc region based on the regional water content  $n_{initial}$  defined in [64] for specimens aged of 58 years old. The finally computed initial water contents of the different disc regions and for the different ages are presented in Table 3.

<b>Age</b>	<b>AO</b>	<b>AI</b>	<b>PO</b>	<b>PI</b>
Less than 18	75.85	79.55	78.25	82.65
36	72.15	75.85	74.55	78.95
58	70.6	74.3	73	77.4
69	69.45	73.75	72.45	76.85
82	68.95	73.25	71.95	76.35

Table 3. Initial water content in each disc region for different ages. Values were obtained by logarithmic fitting of Koeller et al. [21] experimental results where average water content of

the disc were computed for different human specimen ages. The averaged curve was adapted for each region using water content at 58 years old [64].

### 3. Results and discussion

#### 3.1. Age-related stress response changes

In Fig. 4, the computed stress-strain responses of the four regions were plotted for different ages. AO region reveals the highest stress-strain slope and PI region the lowest stress-strain slope. For adolescents (13-18 years) the rigidity is relatively low for the different disc regions. It increases with age until middle age (36 years) and then it becomes almost constant for higher ages with a slight increase from 36 to 58 years old. All computed curves are in accordance with previously found experimental rigidity intervals of Ebara et al. [24].

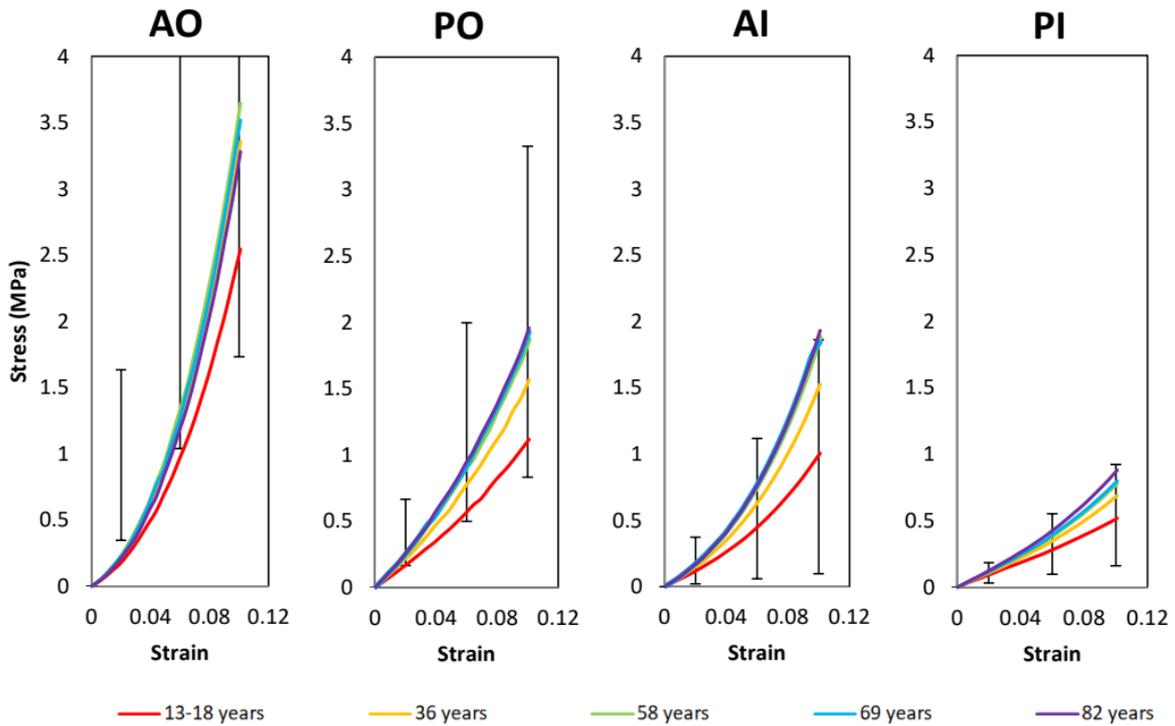
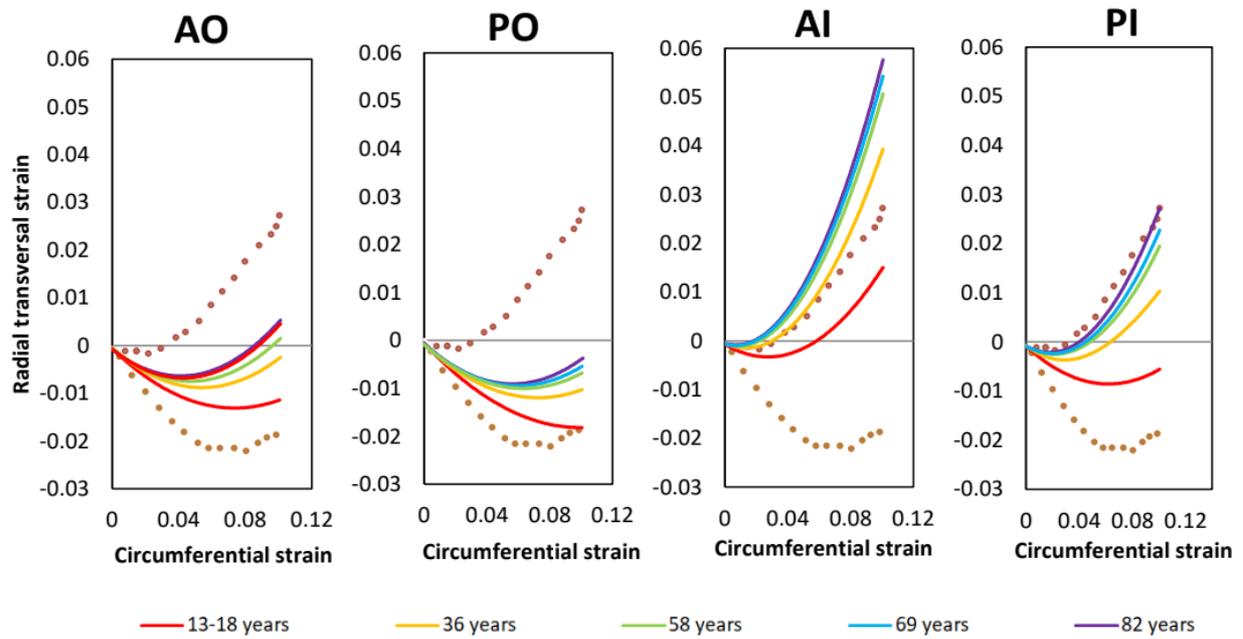


Figure 4. Uniaxial stretching model results for different ages and disc regions compared to the experimental data (including error bars) extracted from [24].

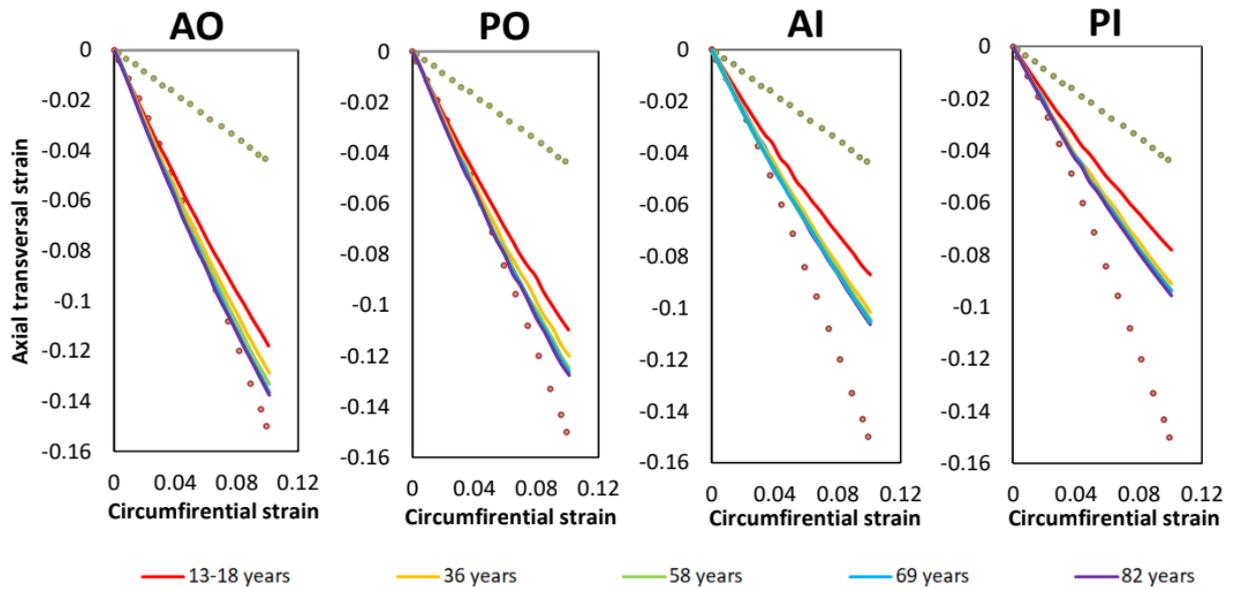
#### 3.2. Age-related transversal response changes

Fig. 5 presents the computed transversal responses of the four regions as a function of the circumferential strain. Radial strains in the outer regions are negative for 13-18 age groups

but they decrease with age presenting very small values (i.e., Poisson's ratio values close to zero). On the other side, the inner regions exhibit an auxetic response with a positive radial strain (i.e., negative Poisson's ratio) at 13-18 years old. Their auxeticity increases while getting older until reaching 36 years old then the effect of age decreases gradually and the values of strains become almost constant. The axial strains of all disc regions demonstrated unusual Poisson's ratio values exceeding 1.0. Their values increase a little bit with age inducing a very small decrease in disc height. Transversal strain results are compared to the typical experimental curves extracted from Balwit et al. [48]. Maximum and minimum values are selected. All predicted responses fail in the experimental limits curves, except AI regions that show higher (auxetic) positive radial strain values.



(A)



(B)

Figure 5. Transversal strain history in radial direction (A) and in axial direction (B) for different ages and disc regions compared to the upper and lower limits (in symbols) of experimental data extracted from [48].

### 3.3. Local stress-strain fields

Local stress-strain fields are plotted and compared for 13-18 and 82 years of the four annulus regions in Fig. 6. The circumferential stress in the lamellae increases with age for all disc regions except for AI. No change is noticed in the inter-lamellar matrix.

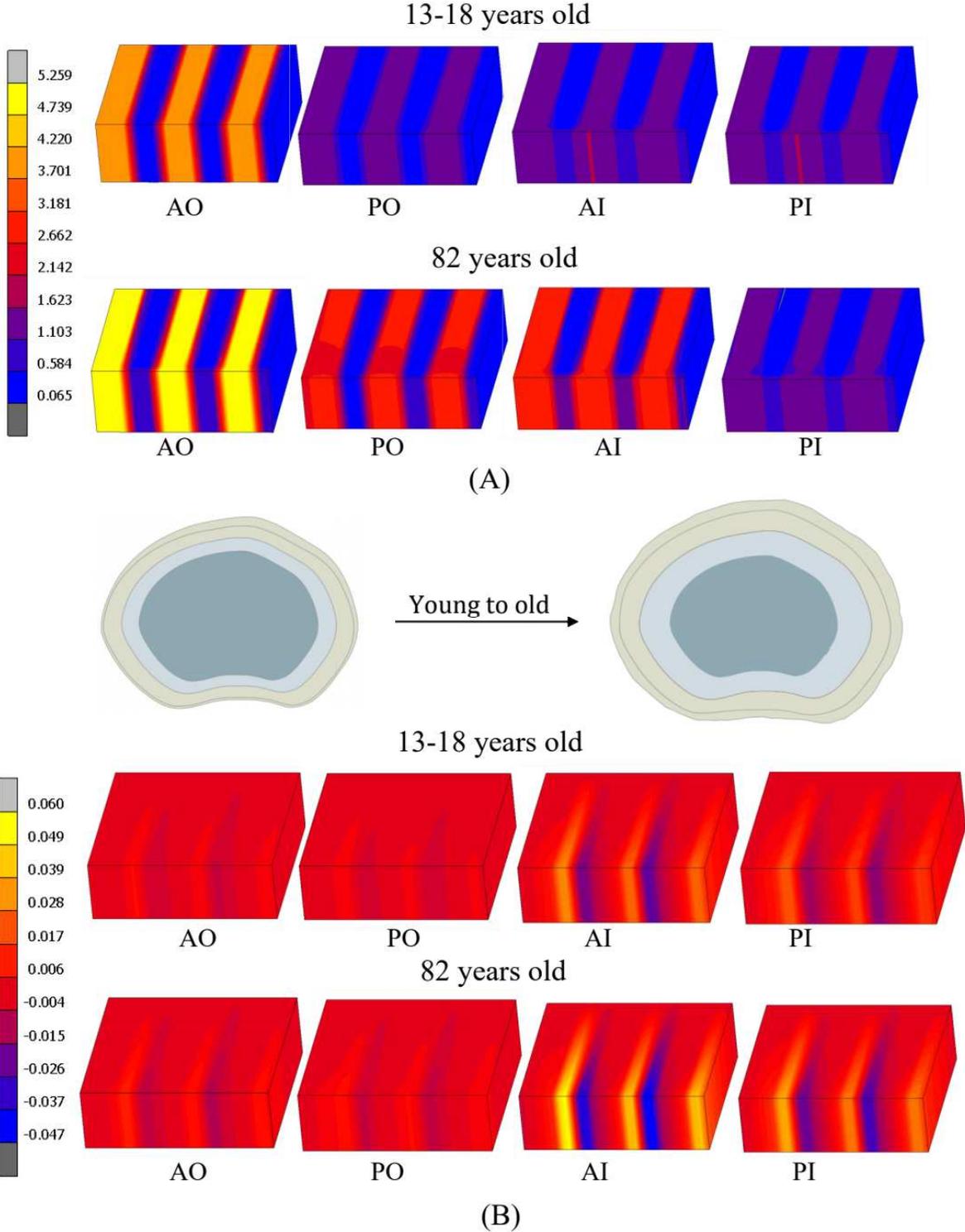


Figure 6. Distribution of the circumferential stress (A) and the shear strain (B) fields for young and old disc tissues along with regional dependency; The sketch shows the interpreted consequences on the disc volumetric change.

The shear strain increases with age in all disc regions with a greater effect in the inner regions where the local values increase by two or three times compared to those of a young disc. The circumferential strain fields were also calculated but not plotted since no effect of age on the local results were observed whatever the regions.

### **3.4. Correlation between intrinsic mechanics of disc and its functionality**

As any other soft tissue, the microstructure components that vary with age inside the intervertebral disc annulus should normally influence its mechanical response. An unexpected result was noticed by many experimental contributions which did not evidence a significant effect of age on the rigidity of the annulus and globally on its mechanical behavior under stretching. For this reason, a microstructure-based modeling approach previously validated for reproducing the multiaxial behavior of the intervertebral disc annulus is used to investigate this issue and to try to figure out the effect of aging on the mechanical behavior of the soft tissue. By varying the oriented fibers amount and the equilibrated water content as a function of the age, the effect of age on the stress-strain and transversal strains was studied and analyzed by means of the model. From the obtained in-silico results, we can notice that the rigidity of the disc is relatively low for young individuals (below 20 years old); then it increases while individuals are getting older until reaching maturity and then it remains almost constant for the rest of their life. It is important to mention that most of the in-vitro experimental tests of the literature are made on specimens of old cadavers because of their availability compared to specimens of young individuals. As an illustrative example, the in-vitro tests of Ebara et al. [24] and Holzapfel et al. [25] were established on specimens extracted from spines of an age varying between 26 to 53 years old, and between 27 and 82 years old, respectively. Weak effect of age was found on the annulus rigidity since most of the experiments are done on cadavers of mature or quasi-mature discs that have an almost stable

stress-strain conduct. This observation was reported by Yammine [65] who mentioned that experimental results available in the literature that were established on human cadaveric are biased toward elderly. Also, we can see from the stress-strain curves in Fig. 4 that the rigidity of the posterior disc regions (PO and PI) are lower than that of anterior disc regions (AO and PO) for the different life stages. The PI region remains the weakest part of the disc with the lowest slope of the stress-strain response and hence the lowest rigidity. This result is in accordance with the medical observations stating that many disc degeneration problems such as hernia often starts from the PI region of the annulus for the different life stages. The transversal strains conducts highlight a very important mechanical parameter that varies with age, that is, the 'Poisson's ratio'. Since the disc is always under quasi-permanent load caused by the body weight, the Poisson's ratio will usually be active and will be a factor affecting the height and diameter of the disc (as illustrated in Fig. 6). Any effect of age on this parameter will directly affect the disc morphology. Despite the major importance of this mechanical property, no previous contributions investigated the aging effect on it. Under controlled circumferential strain, the absolute value of the radial strain describing the change of the annulus width and as a consequence the disc diameter increases largely with age. At the same time, the absolute value of the axial strain affecting disc height slightly increases. Generally, these changes will not influence the global disc height, but it could be apparent in the thinner parts of the disc as the posterior outer region. This would be seen especially for discs of the lowest part of the spinal column where big differences of the anterior and posterior thickness in the unloaded state are often witnessed. This is in line with many experimental observations in the literature where we could see that the diameter highly increases with age [21, 66] causing in some cases disc bulging problems that were found to be correlated with age [2]. As well, although the disc is known to lose a part of its hydration, the healthy annulus height affected by only microstructure changes is found not to change a lot with age [21, 66-67]. The

positive strain in the radial direction (equivalent to a negative Poisson's ratio) indicates the tissue swelling in this direction and shows an auxetic effect. The axial transversal strain values are very close from the applied circumferential strain (equivalent to a Poisson's ratio between 0.7 and 1). These Poisson's ratios are unusual since the normal range of this material property is between 0 and 0.5 which points out an extraordinary response of this soft tissue allowing it to accomplish its damping function under the different applied external mechanical loads. With age, we notice that the values get farther from the normal values which could affect the disc functionality and decreases its resistance. Seeking for more explanations about the effect of age on the different zones of annulus lamellar structure, local numerical stress and strain fields of the different disc regions are plotted and analyzed in Fig. 6. With age the circumferential stresses increase in the lamellae while remaining constant in the inter-lamellar matrix. The strain field in the same direction were constant for the different ages highlighting the stiffening of the lamellae induced by the microstructure changes. All the disc lamellar zones undergo noticeable stiffening except for PI which makes it the weakest part of the disc and increases the risk of injury initiation inside it under high mechanical loads. The observation of local strain fields shows that the shear strains are higher in the inner parts of the disc. Their amounts increase with age especially in the lamellar/inter-lamellar transition zones alerting for a high risk of delamination initiation. Great resemblances are identified between the aging effect and hyper-osmotic effect reported by recent experimental contributions [68-70]. Both effects decrease the water content, increase the rigidity and affect the annulus Poisson's ratios in a close manner. These common points open the way to the use of the hyper-osmotic conditions as an alternative accelerating aging method.

### **3.5. Remarks**

The effect of age on disc tissues has been considered in numerous empirical studies. Over the years, different basic observable macro features (thickness, diameter, cracks, tears, rigidity, degeneration state, etc.) have been collected to obtain comprehensive data as a function of age [1-2, 8-9, 11, 24-25, 66-67]. In parallel, many experimental findings showed that disc microstructural components undergo variations with age. The total collagen fibers (type I and type II), the proteoglycan and water contents change continuously [18, 21]. However, the connection between the macro observations and the local structure-property relationship is still an open issue and reveals many unexplained findings [24-25]. Indeed, even if the local stiffness (that is the tissue resistance to applied deformation) is certainly a fundamental characteristic, no age influence has been reported earlier. Our modeling approach demonstrated that the (anisotropic) volumetric response, translated by the Poisson effect (that is the transversal expansion/contraction), is a key characteristic to consider in the disc mechanics analysis and its age sensitivity. In this regard, the introduction of the inter-lamellar matrix, allowing to capture both chemical sensibility and time-dependency of the annulus transversal response [46], acts as an actuator of the volumetric response. This functionality of the inter-lamellar matrix is not considered in previous models. Stiffness and volumetric behavior are directly correlated to the solid (ECM and fibers) and fluid phases that evolve with age as reported by many experimental studies [18, 21]. Behind the microstructure evolution is hiding biological events interacting in a complex manner with the disc human annulus disc response. According to the challenging task of age effect prediction, our approach takes into account the mechano-biochemical interactions but indirectly through the material kinetics with age. The use of comprehensive relationships between constituents and local properties allows to provide a quantitative prediction of the age-sensitive mechanics of the healthy disc along with regional dependency.

### **3.6. Limitations**

It is worth noticing that our in-silico results were obtained with some restrictions. The fibers and water contents for each age could vary between individuals, gender, disc levels and could be even affected by the testing conditions that change from an experiment to another. The goal of this contribution is to qualitatively identify the effect of aging on the annulus mechanics and to try finding explanations about previously captured in-vitro observations. For this reason and due to the complexity of such study, including age effect on the regional dependency, the average of the fibers and water contents was used for the calculations. The input data were designed using the same disc level in order to avoid any effect of testing or specimen preparation conditions. As well, it is important to mention that each region of the disc does not have an exact value of fibers and water contents inside the disc. They vary gradually in both the circumferential and radial directions. Experimentally, these gradients are very difficult to identify. For this reason, the use of average values from each disc region is often used in this type of studies. Finally, the ECM rigidity is known to alter with age [71-72]. But there is no reliable data available in the literature about the amount of change of this parameter with age from adolescent to elderly. However, we believe that the contribution of the ECM is very small compared to the oriented fibers contribution in the disc stress-strain behavior. For this reason, the same rigidity modulus was used for all ages. This should have a negligible impact on all the results, except the PI stress-strain curves that demonstrate very small rigidity values.

### **4. Conclusion**

The effect of natural aging mechanisms on the human annulus fibrosus mechanics are very complex to identify experimentally. By means of a microstructure-based mechanistic modeling approach, the disc mechanics in terms of rigidity, radial and axial transversal

responses was found to alter going from adolescent to middle age where the disc reaches a certain maturity. After reaching maturity the mechanical properties undergo very slight changes until becoming almost constant with age. For future works, the application of the developed approach on a complete spinal unit structure seems important in order to investigate the age effect on more complex mechanical loads and combined spinal movements.

### **Acknowledgement**

The authors gratefully acknowledge the French ministry of higher education and research for its financial support.

### **References**

- [1] Parkkola, R., Kormano, M., 1992. Lumbar disc and back muscle degeneration on MRI: correlation to age and body mass. *Journal of Spinal Disorders* 5, 86-92.
- [2] Videman, T., Sarna, S., Battie, M.C., Koskinen, S., Gill, K., Paananen, H., Gibbons, L., 1995. The long-term effect of physical loading and exercise lifestyles on back-related symptoms, disability, and spinal pathology among men. *Spine* 20, 699-709.
- [3] Adams, M.A., Hutton, W.C., 1982. Prolapsed intervertebral disc. A hyperflexion injury 1981 Volvo Award in Basic Science. *Spine* 7, 184-191.
- [4] Szilagyi, S.M., Szilagyi, L., Benyo, Z., 2011. A patient specific electro-mechanical model of the heart. *Computer Methods and Programs in Biomedicine* 101, 183-200.
- [5] Lauzeral, N., Borzacchiello, D., Kugler, M., George, D., Rémond, Y., Hostettler, A., Chinesta, F., 2019. A model order reduction approach to create patient-specific mechanical models of human liver in computational medicine applications. *Computer Methods and Programs in Biomedicine* 170, 95-106.
- [6] Razaghi, R., Biglari, H., Karimi, A., 2019. Risk of rupture of the cerebral aneurysm in relation to traumatic brain injury using a patient-specific fluid-structure interaction model. *Computer Methods Programs in Biomedicine* 176, 9-16.
- [7] Roughley, P.J., 2004. Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. *Spine* 29, 2691-2699.
- [8] Pfirrmann, C.W., Metzdorf, A., Elfering, A., Hodler, J., Boos, N., 2006. Effect of aging and degeneration on disc volume and shape: a quantitative study in asymptomatic volunteers. *Journal of Orthopaedic Research* 24, 1086-1094.
- [9] Adams, M.A., Hutton, W.C., 1985. Gradual disc prolapse. *Spine* 10, 524-531.
- [10] Osti, O.L., Vernon-Roberts, B., Moore, R., Fraser, R.D., 1992. Annular tears and disc degeneration in the lumbar spine: A post-mortem study of 135 discs. *The Journal of Bone and Joint Surgery* 74, 678-682.
- [11] Vernon-Roberts, B., Moore, R.J., Fraser, R.D., 2007. The natural history of age-related disc degeneration: The pathology and sequelae of tears. *Spine* 32, 2797-2804.

- [12] Buckwalter, J.A., 1995. Aging and degeneration of the human intervertebral disc. *Spine* 20, 1307-1314.
- [13] Horner, H.A., Urban, J.P.G., 2001. Effect of nutrient supply on the viability of cells from the nucleus pulposus of the intervertebral disc. *Spine* 26, 2543-2549.
- [14] Bibby, S.R., Urban, J.P., 2004. Effect of nutrient deprivation on the viability of intervertebral disc cells. *European Spine Journal* 13, 695-701.
- [15] Adams, M.A., Roughley, P.J., 2006. What is intervertebral disc degeneration, and what causes it? *Spine* 31, 2151-2161.
- [16] Mwale, F., Ciobanu, I., Giannitsios, D., Roughley, P., Steffen, T., Antoniou, J., 2011. Effect of oxygen levels on proteoglycan synthesis by intervertebral disc cells. *Spine* 36, 131-138.
- [17] Malandrino, A., Mak, M., Kamm, R.D., Moeendarbary, E., 2018. Complex mechanics of the heterogeneous extracellular matrix in cancer. *Extreme Mechanics Letters* 21, 25-34.
- [18] Brickley-Parsons, D., Glimcher, M.J., 1984. Is the chemistry of collagen in intervertebral discs an expression of Wolff's Law? A study of the human lumbar spine. *Spine* 9, 148-163.
- [19] Urban, J.P., McMullin, J.F., 1988. Swelling pressure of the lumbar intervertebral discs: influence of age, spinal level, composition, and degeneration. *Spine* 13, 179-187.
- [20] Jenkins, J.P., Hickey, D.S., Zhu, X.P., Machin, M., Isherwood, I., 1985. MR imaging of the intervertebral disc: a quantitative study. *The British Journal of Radiology* 58, 705-709.
- [21] Koeller, W., Funke, F., Hartmann, F., 1984. Biomechanical behaviour of human intervertebral discs subjected to long lasting axial loading. *Biorheology* 21, 675-86.
- [22] Hickey, D.S., Aspden, R.M., Hukins, D.W., Jenkins, J.P., Isherwood, I., 1986. Analysis of magnetic resonance images from normal and degenerate lumbar intervertebral discs. *Spine* 11, 702-708.
- [23] Galante, J.O., 1967. Tensile properties of the human lumbar annulus fibrosus. *Acta Orthopaedica Scandinavica* 100, 1-91.
- [24] Ebara, S., Iatridis, J.C., Setton, L.A., Foster, R.J., Mow, V.C., Weidenbaum, M., 1996. Tensile properties of nondegenerate human lumbar anulus fibrosus. *Spine* 21, 452-461.
- [25] Holzapfel, G.A., Schulze-Bauer, C.A.J., Feigl, G., Regitnig, P., 2005. Single lamellar mechanics of the human lumbar anulus fibrosus. *Biomechanics and Modeling in Mechanobiology* 3, 125-140.
- [26] Schroeder, J., Neff, P., 2003. Invariant formulation of hyperelastic transverse isotropy based on polyconvex free energy functions. *International Journal of Solids and Structures* 40, 401-445.
- [27] Peng, X.Q., Guo, Z.Y., Moran, B., 2005. An anisotropic hyperelastic constitutive model with fiber-matrix shear interaction for the human annulus fibrosus. *Journal of Applied Mechanics* 73, 815-824.
- [28] Balzani, D., Neff, P., Schroeder, J., Holzapfel, G.A., 2006. A polyconvex framework for soft biological tissues: adjustment to experimental data. *International Journal of Solids and Structures* 43, 6052-6070.
- [29] Guo, Z.Y., Peng, X.Q., Moran, B., 2006. A composites-based hyperelastic constitutive model for soft tissue with application to the human annulus fibrosus. *Journal of the Mechanics and Physics of Solids* 54, 1952-1971.
- [30] Klisch, S.M., Lotz, J.C., 2000. A special theory of biphasic mixtures and experimental results for human annulus fibrosus tested in confined compression. *Journal of Biomechanical Engineering* 122, 180-188.
- [31] Riches, P.E., Dhillon, N., Lotz, J., Woods, A.W., McNally, D.S., 2002. The internal mechanics of the intervertebral disc under cyclic loading. *Journal of Biomechanics* 35, 1263-1271.

- [32] Ehlers, W., Karajan, N., Markert, B., 2009. An extended biphasic model for charged hydrated tissues with application to the intervertebral disc. *Biomechanics and Modeling in Mechanobiology* 8, 233-251.
- [33] Barthelemy, V.M.P., van Rijsbergen, M.M., Wilson, W., Huyghe, J.M., van Rietbergen, B., Ito, K., 2016. A computational spinal motion segment model incorporating a matrix composition-based model of the intervertebral disc. *Journal of the Mechanical Behavior of Biomedical Materials* 54, 194-204.
- [34] Chetoui, M.A., Boiron, O., Dogui, A., Deplano, V., 2017. Prediction of intervertebral disc mechanical response to axial load using isotropic and fiber reinforced FE models. *Computer Methods in Biomechanics and Biomedical Engineering* 20, 39-40.
- [35] Soukane, D.M., Shirazi-Adl, A., Urban, J.P.G., 2007. Computation of coupled diffusion of oxygen, glucose and lactic acid in an intervertebral disc. *Journal of Biomechanics* 40, 2645-2654.
- [36] Huang, C.Y., Gu, W.Y., 2008. Effects of mechanical compression on metabolism and distribution of oxygen and lactate in intervertebral disc. *Journal of Biomechanics* 41, 1184-1196.
- [37] Louman-Gardiner, K.M., Coombe, D., Hunter, C. J., 2011. Computation models simulating notochordal cell extinction during early ageing of an intervertebral disc. *Computer Methods in Biomechanics and Biomedical Engineering* 14, 1071-1077.
- [38] Malandrino, A., Noailly, J., Lacroix, D., 2011. The effect of sustained compression on oxygen metabolic transport in the intervertebral disc decreases with degenerative changes. *PLOS Computational Biology* 7, e1002112.
- [39] Zhu, Q., Jackson, A.R., Gu, W.Y., 2012. Cell viability in intervertebral disc under various nutritional and dynamic loading conditions: 3D finite element analysis. *Journal of Biomechanics* 45, 2769-2777.
- [40] Galbusera, F., Mietsch, A., Schmidt, H., Wilke, H.J., Neidlinger-Wilke, C., 2013. Effect of intervertebral disc degeneration on disc cell viability: a numerical investigation. *Computer Methods in Biomechanics and Biomedical Engineering* 16, 328-337.
- [41] Chetoui, M.A., Boiron, O., Ghiss, M., Dogui, A., Deplano, V., 2019. Assessment of intervertebral disc degeneration-related properties using finite element models based on  $\rho_H$ -weighted MRI data. *Biomechanics and Modeling in Mechanobiology* 18, 17-28.
- [42] Zhu, Q., Gao, X., Gu, W., 2014. Temporal changes of mechanical signals and extracellular composition in human intervertebral disc during degenerative progression. *Journal of Biomechanics* 47, 3734-3743.
- [43] Zhu, Q., Gao, X., Chen, C., Gu, W., Brown, M.D., 2020. Effect of intervertebral disc degeneration on mechanical and electric signals at the interface between disc and vertebra. *Journal of Biomechanics* 104, 109756.
- [44] Wills, C.R., Malandrino, A., van Rijsbergen, M.M., Lacroix, D., Ito, K., Noailly, J., 2016. Simulating the sensitivity of cell nutritive environment to composition changes within the intervertebral disc. *Journal of the Mechanics and Physics of Solids* 90, 108-123.
- [45] Derrouiche, A., Zaïri, F., Zaïri, F., 2019. A chemo-mechanical model for osmo-inelastic effects in the annulus fibrosus. *Biomechanics and Modeling in Mechanobiology* 18, 1773-1790.
- [46] Kandil, K., Zaïri, F., Derrouiche, A., Messenger, T., Zaïri, F., 2019. Interlamellar-induced time-dependent response of disc annulus: a microstructure-based chemo-viscoelastic model. *Acta Biomaterialia* 100, 75-91.
- [47] Kandil, K., Zaïri, F., Messenger, T., Zaïri, F., 2020. Interlamellar matrix governs human annulus fibrosus multiaxial behavior. *Scientific Reports*.

- [48] Baldit, D., Ambard, D., Cherblanc, F., Royer, P., 2014. Experimental analysis of the transverse mechanical behaviour of annulus fibrosus tissue. *Biomechanics and Modeling in Mechanobiology* 13, 643-652.
- [49] Derrouiche, A., Karoui, A., Zaïri, F., Ismail, J., Qu, Z., Chaabane, M., Zaïri, F., 2020. The two Poisson's ratios in annulus fibrosus: relation with the osmo-inelastic features. *Mechanics of Soft Materials* 2, 1.
- [50] Bibby, S.R.S., Jones, D.A., Ripley, R.M., Urban, J.P., 2005. Metabolism of the intervertebral disc: effects of low levels of oxygen, glucose, and pH on rates of energy metabolism of bovine nucleus pulposus cells. *Spine* 30, 487-496.
- [51] Inoue, H., Takeda, T., 1975. Three-dimensional observation of collagen framework of lumbar intervertebral discs. *Acta Orthopaedica* 46, 949-956.
- [52] Eyre, D.R., 1979. Biochemistry of the intervertebral disc. *International Review of Connective Tissue Research* 8, 227-291.
- [53] Cassidy, J.J., Hiltner, A., Baer, E., 1989. Hierarchical structure of the intervertebral disc. *Connective Tissue Research* 23, 75-88.
- [54] Yu, J., Tirlapur, U., Fairbank, J., Handford, P., Roberts, S., Winlove, C.P., Cui, Z., Urban, J., 2007. Microfibrils, elastin fibres and collagen fibres in the human intervertebral disc and bovine tail disc. *Journal of Anatomy* 210, 460-471.
- [55] Gent, A.N., 1996. A new constitutive relation for rubber. *Rubber Chemistry and Technology* 69, 59-61.
- [56] Gurtin, M.E., Anand, L., 2005. The decomposition  $F = FeFp$ , material symmetry, and plastic irrotationality for solids that are isotropic-viscoplastic or amorphous. *International Journal of Plasticity* 21, 1686-1719.
- [57] Bergstrom, J.S., Boyce, M.C., 1998. Constitutive modeling of the large strain time-dependent behavior of elastomers. *Journal of the Mechanics and Physics of Solids* 46, 931-954.
- [58] Cantournet, S., Boyce, M.C., Tsou, A.H., 2007. Micromechanics and macromechanics of carbon nanotube-enhanced elastomers. *Journal of the Mechanics and Physics of Solids* 55, 1321-1339.
- [59] Adam, C., Rouch, P., Skalli, W., 2015. Inter-lamellar shear resistance confers compressive stiffness in the intervertebral disc: an image-based modelling study on the bovine caudal disc. *Journal of Biomechanics* 48, 4303-4308.
- [60] Mengoni, M., Luxmoore, B.J., Wijayathunga, V.N., Jones, A.C., Broom, N.D., Wilcox, R.K., 2015. Derivation of inter-lamellar behaviour of the intervertebral disc annulus. *Journal of the Mechanical Behavior of Biomedical Materials* 48, 164-172.
- [61] Tavakoli, J., Elliott, D.M., Costi, J.J., 2016. Structure and mechanical function of the inter-lamellar matrix of the annulus fibrosus in the disc. *Journal of Orthopaedic Research* 34, 1307-1315.
- [62] Ovalle-Rodas, C., Zaïri, F., Naït-Abdelaziz, M., Charrier, P., 2016. A thermo-visco-hyperelastic model for the heat build-up during low-cycle fatigue of filled rubbers: formulation, implementation and experimental verification. *International Journal of Plasticity* 79, 217-236.
- [63] Guo, Q., Zaïri, F., Guo, X., 2018. A thermo-viscoelastic-damage constitutive model for cyclically loaded rubbers. Part I: model formulation and numerical examples. *International Journal of Plasticity* 101, 106-124.
- [64] Iatridis, J.C., MacLean, J.J., O'Brien, M., Stokes, I.A., 2007. Measurements of proteoglycan and water content distribution in human lumbar intervertebral discs. *Spine* 32, 1493-1497.
- [65] Yammine, K., 2019. Published human cadaveric measurements are strongly biased toward the elderly population. *Clinical Anatomy* 33, 804-808.

- [66] Twomey, L., Taylor, J., 1988. Age changes in the lumbar spinal and intervertebral canals. *Paraplegia* 26, 238-49.
- [67] Amonoo-Kuofi, H.S., 1991. Morphometric changes in the heights and anteroposterior diameters of the lumbar intervertebral discs with age. *Journal of Anatomy* 175, 159-168.
- [68] Derrouiche, A., Zaouali, A., Zaïri, F., Ismail, J., Chaabane, M., Qu, Z., Zaïri, F., 2019b. Osmo-inelastic response of the intervertebral disc. *Proceedings of the Institution of Mechanical Engineers. Part H: Journal of Engineering in Medicine* 233, 332-341.
- [69] Derrouiche, A., Feki, F., Zaïri, F., Taktak, R., Moulart, M., Qu, Z., Ismail, J., Charfi, S., Haddar, N., Zaïri, F., 2020. How pre-strain affects the chemo-torsional response of the intervertebral disc. *Clinical Biomechanics* 76, 105020 (2020).
- [70] Feki, F., Taktak, R., Kandil, K., Derrouiche, A., Moulart, M., Haddar, N., Zaïri, F., Zaïri, F., 2020. How osmoviscoelastic coupling affects recovery of cyclically compressed intervertebral disc. *Spine* 45, 160553.
- [71] Cortes, D.H., Han, W.M., Smith, L.J., Elliott, D.M., 2013. Mechanical properties of the extra-fibrillar matrix of human annulus fibrosus are location and age dependent. *Journal of Orthopaedic Research* 31, 1725-1732.
- [72] Stewart, D.M., Monaco, L.A., Gregory, D.E., 2017. The aging disc: using an ovine model to examine age-related differences in the biomechanical properties of the intralamellar matrix of single lamellae. *European Spine Journal* 26, 259-266.