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Gilles Dusfour, Dominique Ambard, Patrick Cañadas, S. Lefloch. Residual strains estimation in the annulus fibrosus through digital image correlation. 2021. hal-03058316v3

HAL Id: hal-03058316

<https://hal.science/hal-03058316v3>

Preprint submitted on 7 Jul 2021 (v3), last revised 25 Nov 2021 (v4)

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Residual strains estimation in the *annulus fibrosus* through digital image correlation.

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Back pain is now among the most prevalent health issues and generally originates from lesions in the annulus fibrosus (AF). While the AF *ex vivo* mechanical properties are increasingly well understood, *in vivo* data are still missing. In particular, very few studies have precisely measured the residual strains within the AF and thus the *in vivo* deformation state of the AF is still misinterpreted and misevaluated. In this work, we propose an original and robust method for quantification of AF residual strains using digital image correlation technics. Ten pig AFs were extracted from adjacent vertebrae followed by a radial incision to release the residual strains. The operations were filmed and then analyzed with custom digital image correlation software in order to quantify the circumferential, radial, and shear residual deformations. Our results show that residual strains are of the same order of magnitude as those *in vivo*. The average circumferential strains are in tension on the outer periphery ([3.32;5.94]%) and in compression on the inner periphery ([-6.4;-1.69]%). The mean radial residual strains are essentially in compression ([-10.4; 2.29]%). Locally, radial and circumferential residual strains can reach very high values of compression, up to 40%. The mean shear strains remain very small ($-0.04\% \pm 2.88\%$). This study also shows that circumferential and radial residual strains evolve linearly along the radius and nonlinearly along the angle. We propose a simple model to predict their spatial variations. Our results and methods will allow the quantification of more realistic *in vivo* strains and stresses within the human intervertebral disc.

Keywords annulus fibrosus ; soft tissue ; intervertebral disc; residual strain; mechanical characterization; growth; digital image correlation

1 Introduction

Back pains is the most prevalent health issue according to the work of (Breivik et al. 2006), and in many cases can lead to cessation of professional activity and psychological conditions like depression and anxiety (McWilliams et al. 2004). Back pain is mainly due to deteriorated or herniated intervertebral discs (IVDs) where the fibrocartilaginous cells struggle to recreate annulus fibrosus (AF) tissue. However, numerical models of the IVD that take into account mechanical and nutritional factors highlight several possible scenarios of annulus fibrosus degeneration (Malandrino et al. 2011). While the *ex-vivo* mechanical behavior of the AF is well documented, very little is known about the *in-vivo* mechanical state. One of the more important factors in realistic models of the *in-vivo* state is accurate identification of the residual strain fields, as has already been done in arteries (Taber et al. 2001).

The most commonly used technique to highlight residual strains is to make one or several incisions within the tissue and to observe its opening. Once the tissue is fully open, the residual strains are estimated through *opening angle method* (Fung 1984). Broad approximations are made as to the roundness of the studied tissue, which lead to global and sometimes poor estimations of the residual strain fields. Recently, (Sigaeva et al. 2019) made improvements of the *opening angle method* in order to apply it to different parts of the same tissue. Such improvements allow several tissue geometries to be taken into account, including many cylindrical shapes, enabling the modeling of residual strains in asymmetric geometries. Estimations of residual strains and stresses via radial incisions have been densely reported in the literature for cardiovascular tissues such as arteries and heart (Omens et al. 2003; Labrosse et al. 2013), but little is known about the residual strains in conjonctive tissues like the AF. To date, only (Michalek et al. 2012), (Duclos et al. 2017) and (Mengoni et al. 2017) have estimated the residual strains in the AF of bovine caudal IVDs. These studies are based on the *opening angle method* and the *arc length method* to obtain the circumferential strains, whereas the radial strains are estimated by following the evolution of lamellae thickness during the stress relaxation experiment. However, the estimation of global residual strains in these studies is based on the roundness of the tissue, which complicates reproduction of this technique on the more complex geometry of human AF. Noteworthy, (Duclos et al. 2017) also used polarization birefringence microscopy to estimate the fiber crimped period before and after the radial incision. This method shows circumferential residual strains qualitatively equivalent to the arc length method but quantitatively different. Therefore, we decided to realize a full field estimation of the residual strains

through digital image correlation (DIC) techniques and to apply this to the pig models more commonly used in the IVD biomechanics community (Busscher et al. 2010; Yingling et al. 1999).

Residual strains are present in a large variety of biological tissues in both animal and plant species (Goriely 2017). However, their genesis and purpose have not been clearly identified. Many authors consider differential growth within organs to be responsible for residual strains (Goriely 2017; Zahn et al. 2018). Others hypothesize that gradients in mechanical properties or in negatively charged molecules, such as proteoglycans, play a major role in the generation of residual strains and stresses (Azeloglu et al. 2008; Yang et al. 2018). For (Lanir 2009), residual strains and stresses are the result of all of the above mechanisms combined. Interestingly, residual strains seem to play a structural role in load-bearing tissues. Indeed, when biological tissues undergoing internal pressure, such as arteries, heart, or AF, are cut in the radial direction they tend to open up (Fung 1991; Michalek et al. 2012; Duclos et al. 2017). Such residual strain or stress fields are assumed to create a homeostatic state of strain or stress in the loaded organs. This hypothesis led to the development of strain-induced growth laws to explain the pattern of residual strains in biological tissues (Taber et al. 2001).

Several attempts have been made to model the development of biological tissues (Goriely 2017; Ambrosi et al. 2002; Lubarda et al. 2002). Interestingly, most of these models have been applied to cardiovascular tissues. To this date no growth model of the intervertebral disc has been proposed. Moreover, very few authors have compared experimental residual strain fields to those studied using simulations, excepting the work of (Taber et al. 2001). In order to completely model such a complex phenomenon, anatomical, nutritional, mechanical, and biological data are required and the results of the simulation finally need to be validated. Many of the incoming data are available in the literature through the work of (Taylor 1975; Maroudas et al. 1975; Peacock 1951; Antoniou et al. 1996). Therefore, it is of crucial importance to accurately evaluate the residual strain fields to both validate and drive future IVD growth laws.

In this study, samples of pig AF were extracted from the adjacent vertebrae and cut in the radial direction. We propose a new method to measure residual strains regardless of the AF shape. The AF opening was recorded with a camera and analyzed through a dedicated DIC software to evaluate the residual strain fields in two dimensions. Novel data on residual strain patterns are reported. We hypothesized that the AFs display heterogeneous and anisotropic residual strain patterns dependent on both radius and angle. Moreover, it is assumed that the residual strain patterns can be modeled in terms of a linear relationship with radius and a nonlinear relationship with angle.

2 Materials and methods

2.1 Specimen preparation

A total of ten AF sheet specimens were harvested from the spine of a three-month-old white pig weighing approximately 30 kg. Cervical (C2-C3, C3-C4, C5-C6, C6-C7), thoracic (T1-T2, T2-T3, T3-4, T10-T11, T11-T12), and lumbar (L1-L2, L6-S) AFs were extracted. The spine was obtained from a local slaughter house and immediately frozen at -20°C in a physiological solution of NaCl (9g/L). Prior to extracting the disc, the spinous and transverse processes were cut using a saw. The discs were separated from the adjacent vertebral bodies with a homemade double blade cutter to obtain a planar AF sheet of 1mm thickness. Following extraction, the AF was immediately cleaned with a physiological solution and graphite powder was deposited to enhance contrast. The samples were then immersed in a physiological solution of NaCl (9g/L) at 37°C in an aluminum container with a glass window on the bottom. This glass allowed images to be captured from below while making a radial incision with a scalpel on the upper lateral side of the AF (Fig 2). The images were captured with a camera (IDS-UI-3360-CP-M-GL Rev2, Fujifilm lens Fujinon-1:1.4/16mm) at an acquisition frequency of 1 Hz during the radial incision and 0.1Hz for 4 hours during the residual strain relaxation period. The entire experimental setup is shown in Figure 1.

2.2 Numerical sample generation

For each experiment, a numerical geometry and a hexaedric mesh of the open sample were created with the GMSH mesh generator (Geuzaine et al. 2009). The boundaries of the numerical sample were defined manually to avoid any side effects. For each sample, the center point of the nucleus pulposus was determined with contour detection using the *open cv2* python library. This point allowed us to compute an angle for each node of the mesh and to identify whether the node belonged to the anterior [0° - 60°], lateral [60° - 120°], or posterior [120° - 180°] quadrant as illustrated on Fig 3(b). This calculation can be extended for any division of the AF between two angles [α_{min} ; α_{max}]. Such a division allows a refinement of the analysis. Finally, a thermal calculation allowed us to compute a normalized radius as well as radial and circumferential direction for each node of the mesh (Figs 3(a), 3(c), 3(d)).

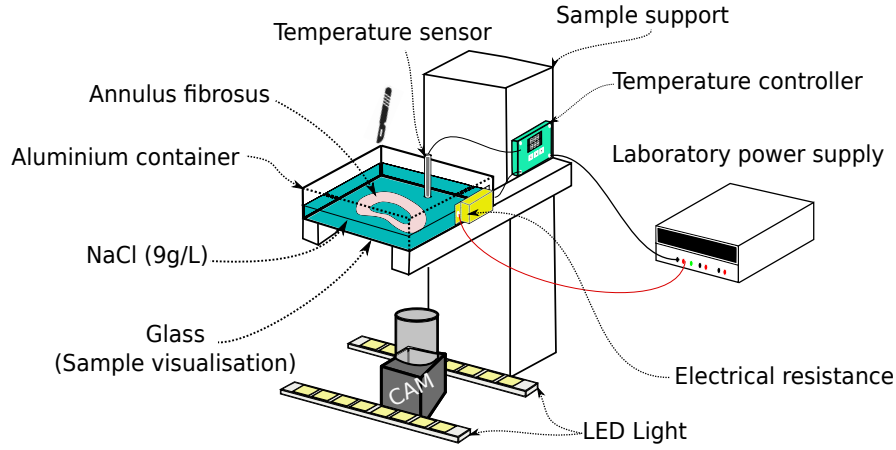


Figure 1: Image acquisition setup used to acquire the images during the relaxation process

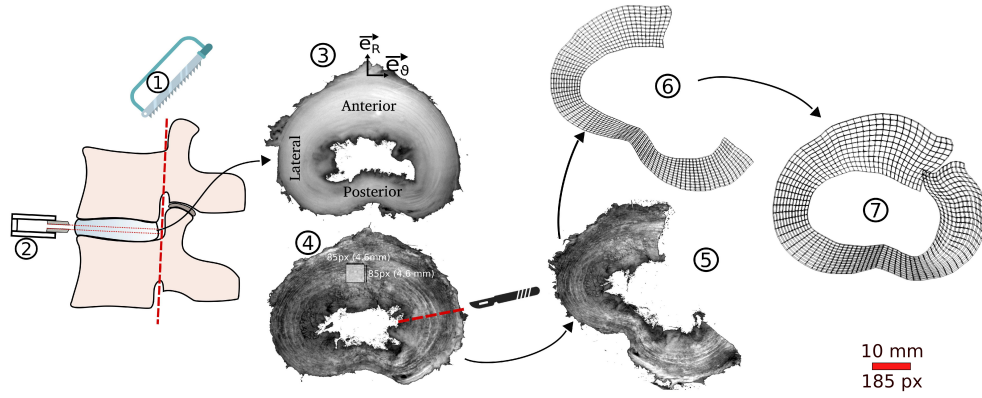


Figure 2: Schematic of the sheet specimen extraction protocol. Spinous and transverse processes are cut with a saw, the sheet of AF is obtained with a homemade double blade cutter, and the outer and inner specimens are extracted with a surgical knife. The DIC windows size is depicted in stage 4. A scale bar is provided in the bottom right of the figure.

2.3 Image analysis

2.3.1 Digital Image correlation

In order to estimate the residual strain fields, an iterative DIC technique was implemented in *python 2.7*. For each sample, a total of 30 images were analyzed with a mean number of 3480 ± 2427 correlation points. For each point, a subset dimension of 85×85 pixels² (2.14×2.14 mm²) was searched in a 150×150 px² (8.1 mm²) research area to identify both the displacement and the rotation of the subset. The research area in the deformed image was centered on the coordinates of the correlation point of the reference image. An initial angle interval of $\pm 30^\circ$ was chosen. For each correlation point, if the correlation criterion (see eq 1) was higher than 0.9 the reference and deformed coordinates as well as the correlation criterion and the rotation angle were saved in a results array. Otherwise, another step of correlation was launched and an initial guess for the displacement and the rotation of the remaining points was computed using the mean displacement of the neighboring points already correlated. If needed, the angle interval was enlarged during the computation to maximize the correlation criterion.

2.3.2 Correlation criterion

The zero-normalized cross-correlation criterion (ZNCC), as proposed by (Pan et al. 2009b), was used to estimate the displacement of each point in between the reference image f and the deformed image g .

$$r_{ij} = \frac{\sum_{m \in M} \sum_{n \in N} [f(m, n) - \bar{f}][g(m + i, n + j) - \bar{g}]}{\sqrt{\sum_{m \in M} \sum_{n \in N} [f(m, n) - \bar{f}]^2} \sqrt{\sum_{m \in M} \sum_{n \in N} [g(m + i, n + j) - \bar{g}]^2}} \quad (1)$$

Where M and N are the x and y intervals of the subset domain. The mean grayscale value of the reference and deformed image are represented by \bar{f} and \bar{g} , respectively. The displacement in the x and y directions are determined by:

$$(\Delta x, \Delta y) = \underset{(i,j)}{\operatorname{argmax}} \{r\} \quad (2)$$

2.3.3 Pointwise least square for strain estimation

In order to obtain full-field strain distribution from a noisy full-field displacement estimation, a pointwise local least-squares fitting technique was used as proposed by (Wattrisse et al. 2001) and (Pan et al. 2009). The implementation of this technique requires, for each node of the mesh, the creation of a circular window (arbitrary radius of 40 px) and to extract all the correlation points in the neighboring pixels. The displacement distribution in this window is then approximated as a linear plane:

$$\begin{aligned} u(i, j) &= a_0 + a_1x + a_2y \\ v(i, j) &= b_0 + b_1x + b_2y \end{aligned} \quad (3)$$

Where, $u(i,j)$ and $v(i,j)$ are the original horizontal and vertical displacements at pixel location (i,j) . The least-squares method is then used to estimate the local unknown polynomial coefficients. Finally, the imposed displacement for each node of the hexaedric mesh is computed with identified polynomial coefficients and node coordinates. The strains are then computed at each Gauss Point element, through the derivative of the form function from the finite element technique, and extrapolated at each node location.

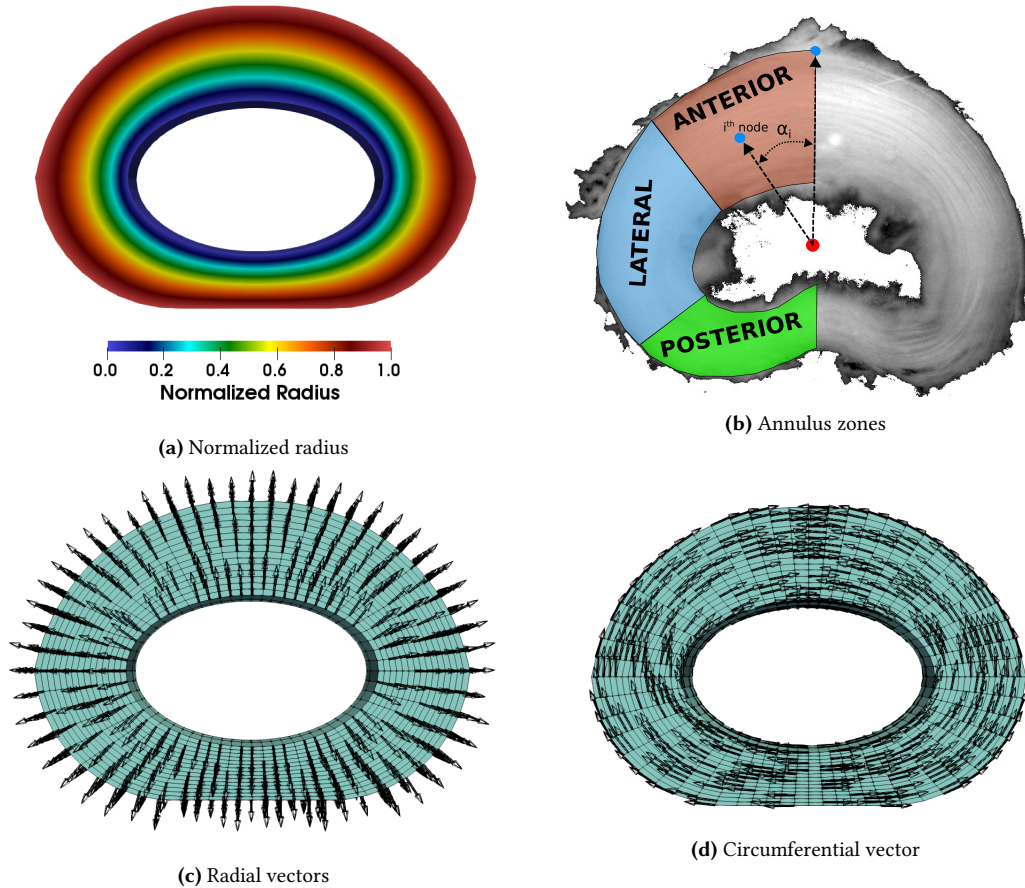


Figure 3: Numerical model parameters. Normalized radius (a), Radial (c) and circumferential vectors (d) were estimated using a thermal flux (see section 2.2). Anterior, lateral and posterior quadrants were determined by their specific angles from the antero-posterior central line

2.4 Radial and circumferential strain field estimation

For each node of the final closed mesh, the Eulerian strain tensor $\underline{\underline{e}}$ is projected on both radial (\vec{e}_{RR}) and circumferential ($\vec{e}_{\theta\theta}$) directions (see figures 3(c) and 3(d)) to derive radial (e_{RR}), circumferential ($e_{\theta\theta}$) and shear strains ($e_{\theta R}$), with :

$$e_{\theta\theta} = \underline{\underline{e}} : (\vec{e}_{\theta\theta} \otimes \vec{e}_{\theta\theta}) \quad (4)$$

$$e_{RR} = \underline{\underline{e}} : (\underline{\underline{\vec{e}}}_{RR} \otimes \underline{\underline{\vec{e}}}_{RR}) \quad (5)$$

$$e_{\theta R} = \underline{\underline{e}} : (\underline{\underline{\vec{e}}}_{\theta\theta} \otimes \underline{\underline{\vec{e}}}_{RR}) \quad (6)$$

In order to facilitate the reading of residual strain fields, we created graphics to visualize the average evolution of residual strains according to the normalized radius and area of study. Therefore, the radius was divided into 12 equal intervals with a $\frac{1}{12}$ step. For each radius interval $[r_{min}; r_{max}]$ and each zone interval $[\alpha_{min}; \alpha_{max}]$, the median and interquartile range of residual strain were computed.

2.5 Residual strain model fitting

In order to take advantage of such rich residual strain fields, we derive a simple model to fully describe the residual strain state as a function of the local coordinates. The heterogeneous residual strain pattern of the AF was modeled by a linear function of the normalized radius.

$$e_{\theta\theta}(r) = K_{\theta\theta}(\alpha) \times r + O_{\theta\theta}(\alpha) \quad (7)$$

$$e_{RR}(r) = K_{RR} \times r + O_{RR} \quad (8)$$

$$e_{\theta R}(r) = K_{\theta R} \times r + O_{\theta R} \quad (9)$$

Here K_{ii} is the gradient of residual strain along the normalized radius and O_{ii} the offset of the residual strain for the zero normalized radius. In order to respect the strain continuity at 0° and 180° , the gradient $K_{\theta\theta}$ and the initial offset $O_{\theta\theta}$ of the circumferential strain were modeled as the sum of *cosinus* functions with respect to the normalized angle:

$$K_{\theta\theta}(\alpha) = k + \sum_{i=1}^n A_i \cos(i\alpha) \quad (10)$$

$$O_{\theta\theta}(\alpha) = k + \sum_{i=1}^n A_i \cos(i\alpha) \quad (11)$$

Fitting was performed using *python 2.7* and the *curvefit* function from the *scipy.optimize* library. The solution of the *curve_fit* function minimizes the squared error between the predicted and the actual data.

The goodness of the fit between the predicted and measured residual strain (e) was evaluated with the normalized root mean square error (NRMSE) :

$$NRMSE = \frac{\sqrt{\frac{\sum_{i=1}^n (e_i^{num} - e_i^{exp})^2}{n}}}{e_{max}^{exp} - e_{min}^{exp}} \times 100\% \quad (12)$$

2.6 Statistical analysis

The numerical data are presented as a median \pm interquartile range (IQR). All statistical analyses were performed at 95% confidence level using the statistical environment R. Nonparametric tests had to be applied because normality tests (Shapiro–Wilk) showed that none of the data groups have normal distributions. The studied values are the radial strain (e_{RR}), the circumferential strain ($e_{\theta\theta}$), the shear strain ($e_{\theta R}$), the gradient of each strain along the radius (K_{ii}) and the initial offset (O_{ii}). We performed a Kruskal-Wallis test to assess the dependence of the radial strain (e_{RR}), the circumferential strain ($e_{\theta\theta}$) and the shear strain ($e_{\theta R}$) on the normalized radius. Kruskal-Wallis tests were also performed on the gradient of residual strain (K_{ii}) and on the initial offset (O_{ii}) to assess their dependence on the normalized angle.

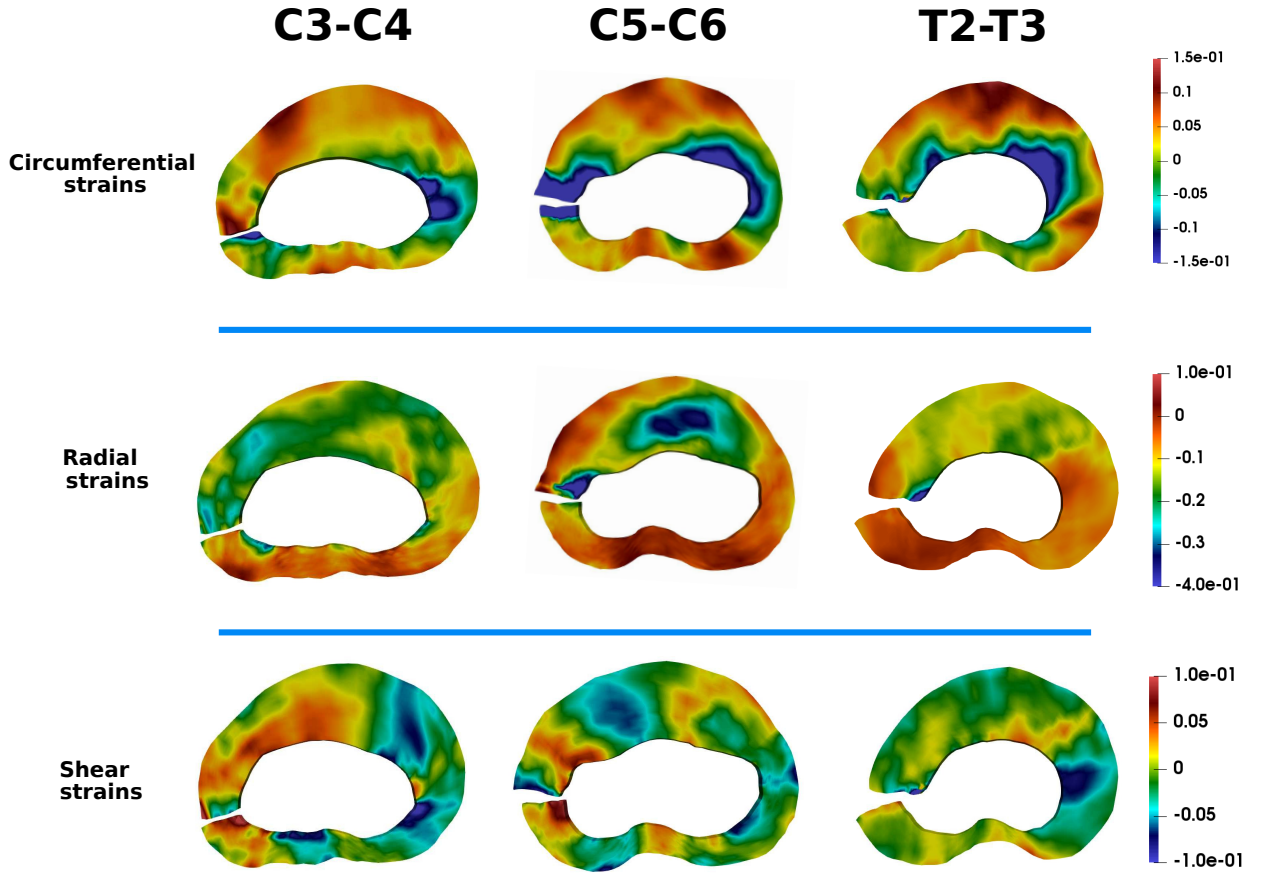


Figure 4: Circumferential, radial and shear residual strain fields for AFs.

3 Results

3.1 Qualitative method results

After the incision, all the samples open up to release the residual strains. These results highlight circumferential tension in the outer AF and compression in the inner AF. Figure 4 shows residual strains fields for three different AFs. Qualitatively, these results suggest that the strain fields are widely heterogeneous and anisotropic. Indeed the circumferential residual eulerian strains ($e_{\theta\theta}$) can exceed 15% in both tension and compression while the radial residual strains (e_{RR}) can reach 40% in compression but rarely go into tension. Clearly, the radial (e_{RR}), circumferential ($e_{\theta\theta}$) and shear ($e_{R\theta}$) residual strains present different pattern highlighting the anisotropic residual strain fields. Moreover, we note that both circumferential ($e_{\theta\theta}$) and radial (e_{RR}) strains vary smoothly with radius and angle but also with disc level. Finally, the shape of the final AF presented in figure 4 highlights the impaired ability of the DIC software to fully close the incisions.

3.2 Comparison to literature

In order to facilitate analysis and to compare our results with the literature (Duclos et al. 2017), the regional (anterior, lateral and posterior) variation along the normalized radius was averaged and is presented in the figures 5(a), 5(c) and 5(b).

Figure 5(a) presents the quantitative data of the circumferential ($e_{\theta\theta}$) residual strains along the normalized radius for the anterior, lateral and posterior regions. The exterior periphery ($r = 1$) is in tension with a more pronounced tensile state on the anterior and posterior quadrant than in the lateral quadrant (respectively $5.94\% \pm 1.54$, $5.48\% \pm 3.06$ and $3.32\% \pm 6.65$). These strains gradually decrease along the radius, getting closer to the zero strain state for the median radius ($r = 0.5$, respectively $2.26\% \pm 4.94\%$, $-1.79\% \pm 5.56\%$ and $-0.12\% \pm 5.25\%$) and finally reach a global large compression state at the inner periphery ($r = 0$, respectively $-6.4\% \pm 13.71\%$, $-6.33\% \pm 11.09\%$, $-1.69\% \pm 9.24\%$). We note the decreasing IQR with increasing radius. Importantly, the Kruskal Wallis test shows that the circumferential residual strains ($e_{\theta\theta}$) are dependent on the normalized radius ($p < 0.001$).

Quantitative data for the radial (e_{RR}) and shear ($e_{R\theta}$) residual strains along the normalized radius are presented in Figs.5(b) and 5(c), respectively. Like the circumferential strains ($e_{\theta\theta}$), the Kruskal Wallis test showed that the radial strains (e_{RR}) are dependent on the normalized radius ($p < 0.05$) but not the shear residual strains ($e_{R\theta}$). The radial residual strains (e_{RR}) gradually increase in the anterior, lateral and posterior

portions from the inner periphery ($r = 0$, respectively $-10.4\% \pm 9.79\%$, $-6.24\% \pm 10.75\%$ and $-4.99\% \pm 5.55\%$) to the outer periphery ($r = 1$, respectively $-4.76\% \pm 9.07\%$, $-5.77\% \pm 5.33\%$ and $2.29\% \pm 4.97\%$). Finally, the shear residual strains ($e_{R\theta}$) are globally close to the zero strain state ($-0.04\% \pm 2.88\%$).

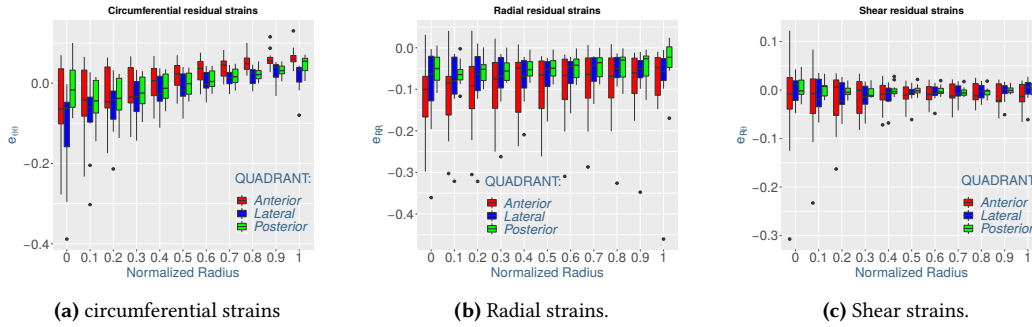


Figure 5: Residual strains in the annulus fibrosus along the normalized radius for three different zones (anterior, lateral and posterior). Closed black circles represent boxplot outliers.

3.3 New local estimation of residual strain

3.3.1 Raw data

Nevertheless, the DIC method provides local estimates allowing us to increase the number of studied portions even with such a large amount of data. This technique allows us to identify significant differences between 15 different portions compartmentalized in 12° angular intervals, starting from the anterior side (0° - 12°) and ending at the posterior side (168° - 180°).

3.3.2 Linear model of residual strain

Figures 6(a),6(c) and 6(e) display the average of all AF residual circumferential, radial and shear strains fields on an idealized pig intervertebral disc. In additions, figures 6(b), 6(d) and 6(f) show overlays of experimental and model-predicted residual strains $e_{\theta\theta}$, e_{RR} and $e_{R\theta}$ versus the normalized radius (r) for 15 different portions and 10 radius values of the idealized pig AF. We note that the IQRs are not presented for reasons of readability. For all the studied discs and zones, the predictions of the circumferential residual strains $e_{\theta\theta}$ agree extremely well with experimental data ($NRMSE = 12.54\% \pm 6.58\%$) while the predictions of the radial (e_{RR}) and shear ($e_{R\theta}$) strains agree less well (respectively $NRMSE = 20.9\% \pm 7.74\%$ and $NRMSE = 21.2\% \pm 7.31\%$).

The first order polynomials model allows us to obtain a gradient and an initial offset of residual strains along the normalized radius for 15 angular portions for all the studied discs. The evolution of the gradients ($K_{i,j}$) and initial offsets ($O_{i,j}$) with respect to the angle for the circumferential, radial and shear residual strains are respectively presented in the figures 7(a),7(b), 7(c),7(d), 7(e) and 7(f) as boxplots representation. While the circumferential gradient ($K_{\theta\theta}$) and initial offset ($O_{\theta\theta}$) evolve in a nonlinear way, both showing a peak centered on the $[48^\circ - 72^\circ]$ interval, the radial and shear gradient (K_{RR} , $K_{R\theta}$) and initial offsets (O_{RR} , $O_{R\theta}$) seem more stable with respect to angle (α).

The results of the Kruskal Wallis test show that the circumferential strains gradient ($K_{\theta\theta}$) and the initial offset ($O_{\theta\theta}$) are dependent on the angular portion (respectively $p < 0.05$ and $p < 0.005$). The Wilcoxon test with a Bonferroni correction highlight that the gradient of the $[48^\circ - 60^\circ]$ and $[60^\circ - 72^\circ]$ portions are significantly higher than the gradient of the $[144^\circ - 156^\circ]$; $[156^\circ - 168^\circ]$ and $[168^\circ - 180^\circ]$ portions ($p < 0.05$). On the other hand the Kruskal Wallis test shows that the gradient and initial offset of the radial (K_{RR} , O_{RR}) and shear ($K_{R\theta}$, $O_{R\theta}$) residual strains are not dependent on the angular portion ($p > 0.05$).

Therefore, the evolution of the circumferential strains gradient ($K_{\theta\theta}$) and initial offset ($O_{\theta\theta}$) are modeled with a triple and double sum of *cosinus* functions, respectively. The parameters of both fit functions are summarized in Table 1. Predictions of circumferential gradient and initial offset with respect to the angle agree well with the experimental data (respectively $NRMSE = 14.19\%$ and $NRMSE = 12.85\%$). Predictions of circumferential gradient and initial offset are plotted in figures 7(a) and 7(b). Regarding the nondependence of radial and shear strains gradient and initial offset on the angle, all of the data have been approximated with a single scalar also presented in table 1.

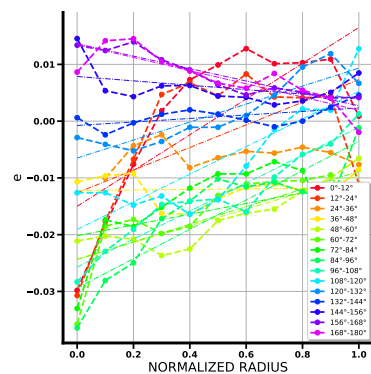
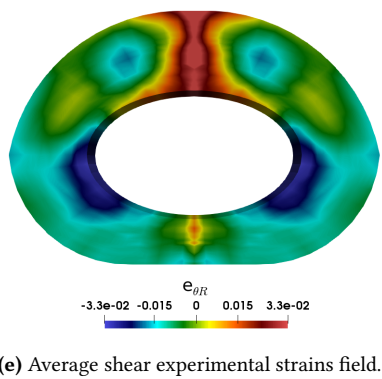
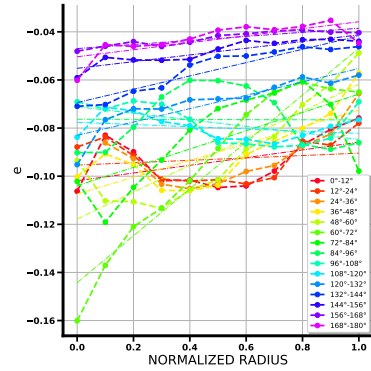
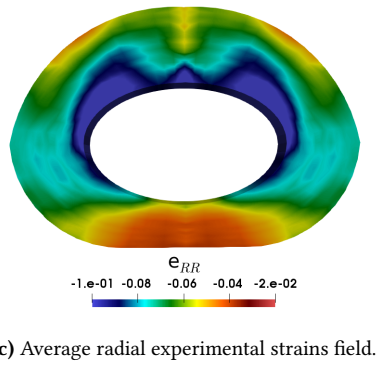
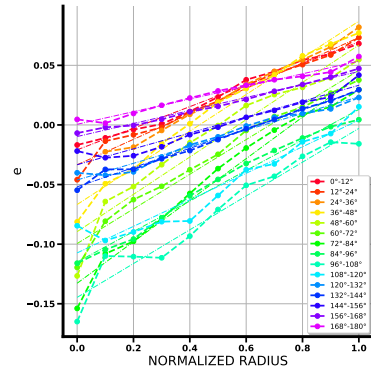
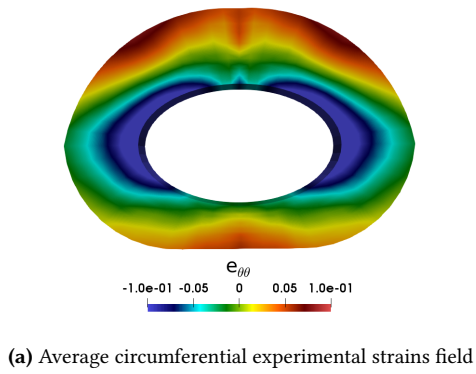


Figure 6: Projection of the average experimental strain fields on an idealized AF and plot of residual strains along the normalized radius for all zones of the AF. In (b), (d) and (f), average experimental data are represented by dotted lines with circle markers and linear model fits are as dash-dotted lines.

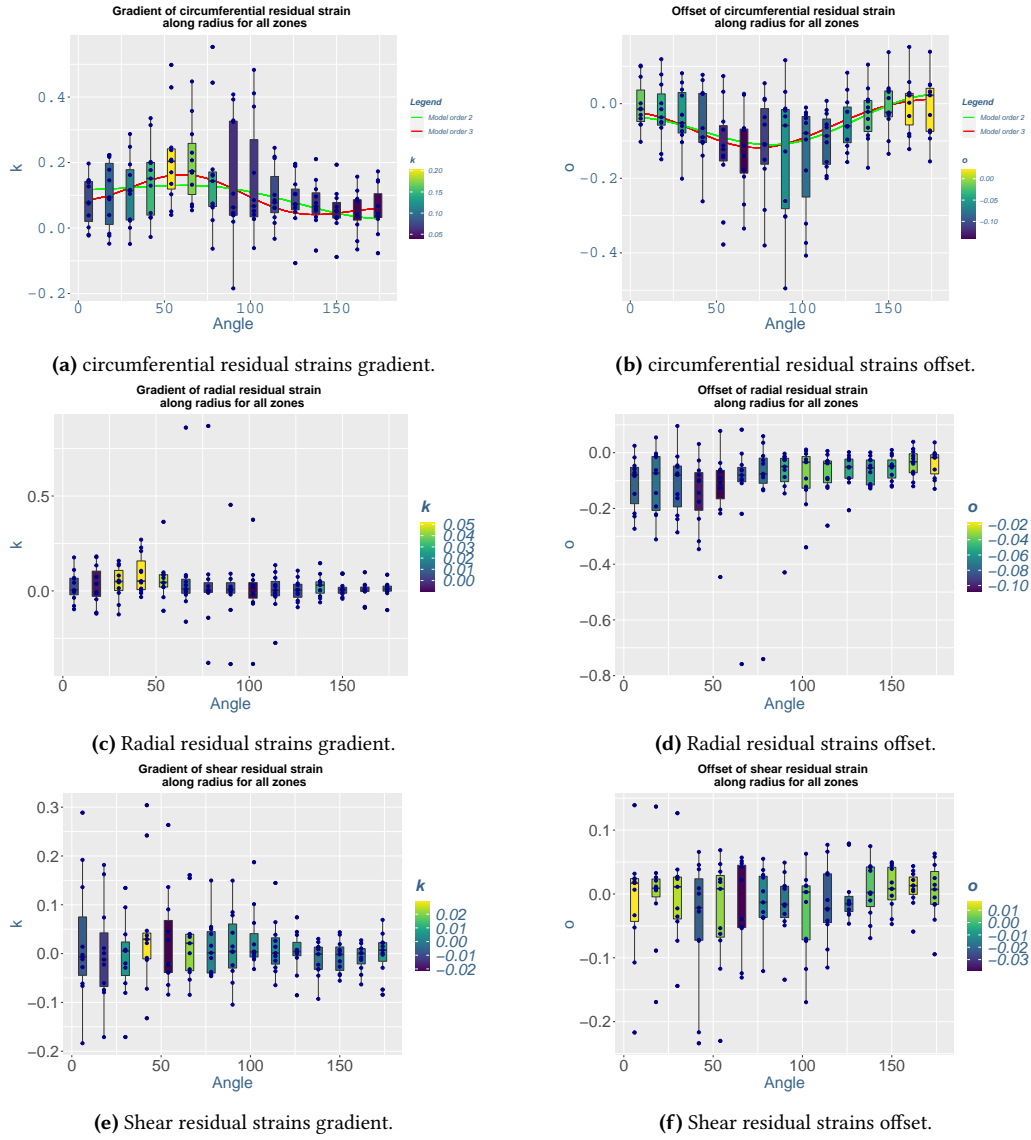


Figure 7: Boxplot of the residual strain gradients and initial offsets along the normalized radius in the circumferential, radial and shear directions. The results of the *cosinus* models are plotted in (a) and (b), showing good agreement with the experimental data.

Parameters	A_1	A_2	A_3	k	NRMSE
$K_{\theta\theta}$	0.045	-0.024	-0.033	0.096	14.19%
$O_{\theta\theta}$	-0.031	0.051	-	-0.057	12.85%
$K_{RR} = 0.012$	-	-	-	-	36.88%
$O_{RR} = -0.063$	-	-	-	-	29.62%
$K_{R\theta} = 0.002$	-	-	-	-	23.12%
$O_{R\theta} = -0.003$	-	-	-	-	28.39%

Table 1: Values of *cosinus* function parameters to describe the evolution of residual strains with respect to the normalized radius and angle.

Finally, the figure 8 shows the linear correlation between the residual strain gradients and the initial offset. Each point represents the residual strain gradient and the initial offset for a portion of a disc. These results establish that the greater the compression of the inner periphery the greater the gradient of residual strain along the normalized radius. (NRMSE: $\theta\theta = 7.47\%$; NRMSE: $RR = 6.88\%$; NRMSE: $R\theta = 7.47\%$)

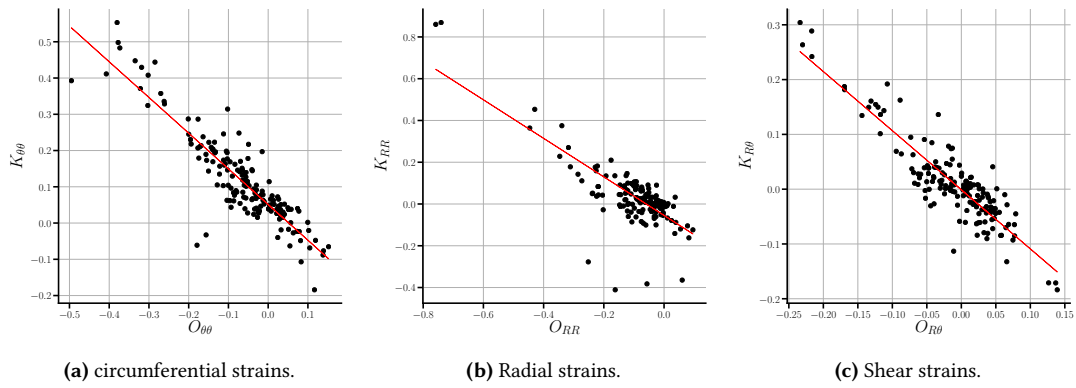


Figure 8: Gradient of residual strains K_{ii} along the normalized radius with respect to the initial offset O_{ii} for each portion of AF of each disc. These figures show the linear relationship between the strain gradient and the intensity of the strains. The more the strains tend towards a negative value on the inner periphery of the AF, the greater the strain gradient along the normalised radius.

4 Discussions

The aim of this study is to develop an original method to locally evaluate residual strains of the pig AF using a digital image correlation technique allowing full field evaluation. In a similar spirit to (Fung 1991) and (Duclos et al. 2017) who respectively cut arteries and bovine AF in the radial direction to release the residual strains, we analyzed residual strains in pig AF and to the best of our knowledge are the first to use DIC to locally estimate the circumferential ($e_{\theta\theta}$), radial (e_{RR}) and shear ($e_{R\theta}$) residual strains in this tissue.

The studies by (Michalek et al. 2012) and (Duclos et al. 2017), realized on bovine AF, reveal similar trends in the distribution of circumferential strains along the normalized radius except for the posterior region. Indeed, whereas the work of (Duclos et al. 2017) presents a minimum of the circumferential strains in the middle zone of the posterior region, our study shows a steady increase from the inner to the outer zone of the AF, in all regions. We find that the amplitude of compressive circumferential strain on the inner zone is half that reported by (Duclos et al. 2017). Moreover, our results reveal a generalized state of compression in the radial direction which is not in accordance with the state of tension in the inner and middle zones presented by (Duclos et al. 2017). These large differences in both amplitude and state of deformation are probably due to the species of the extracted DIV. While our study was realized on pig cervical, thoracic and lumbar AF, the studies of (Michalek et al. 2012) and (Duclos et al. 2017) used bovine tail IVD. Which lends well to this type of experiment because of its roundness and availability, although its morphology is different from the kidney shape of human and pig AF. However, despite showing similar morphology and mechanical behavior, human and pig IVD do not have equivalent dimensions and *in vivo* loading (Busscher et al. 2010; Beckstein et al. 2008). This highlights the necessity to evaluate the residual strain fields in human AF tissue. Therefore, our robust methodology for the estimation of residual strains is of crucial importance regarding the kidney shape of the human AF.

Furthermore, to the best of our knowledge this is the first time that shear residual strains of the AF are reported. Contrary to the circumferential and radial residual strains, the amplitude range of the shear residual strains is very small. The residual strain fields presented in this article, as well as the evolution of residual strains along the radius highlight the relevance of studying the residual strains via the material directions of the AF. Also, radial and tangential strains evolve in a smooth way along the normalized radius. On the contrary, this does not seem to be the case of shear strains. In addition, it has been shown that the residual strains evolve in a linear fashion along the normalized radius with good NRMSE [12% - 21%]. Our analysis highlights significant differences between the various angular portions studied, both in terms of gradient and initial offset of the residual strains. In the case of circumferential strains, the evolution of the gradient and the initial offset are significantly dependent on the angle. Two lateral portions ([48° - 60°] and [60° - 72°]) show gradients that are significantly greater than those of the posterior portions ([144° - 156°], [156° - 168°] and [168° - 180°]), the gradients of which are the lowest in AF. The gradients and the offsets of the radial and shear strains also evolve in a nonlinear way but more experimental data are needed in order to make robust conclusions about the differences between these portions. In a remarkable way, there are locations where the radial and circumferential strains are both positive or both negative, especially in the anterior area. This feature highlights the fact that AF can locally have a negative Poisson's ratio, as already shown by (Dusfour et al. 2020).

There have been many numerical studies in which the authors have attempted to predict the degeneration of the IVD by investigating the coupling between mechanical loading and biological properties (metabolic and nutrient transport, and growth and remodeling through mechanotransduction)

(Huang et al. 2008) and (Malandrino et al. 2011). While most of these studies used mechanical properties found experimentally, none of them considered the residual strain and stress fields as initial conditions of the numerical models. Our results reveal the large amplitude of the medial residual strain distribution along the normalized radius (circumferential: [-6.4% ; 6%], radial : [-10.4%;2.29%] and shear : [-0.04% ± 2.88%]) which could greatly influence the computed global mechanical response as well as the nutrient transport. Such identified residual strain fields would reduce the hoop strains and stresses in the inner periphery of the loaded AF. As a consequence, circumferential strains are likely to be homogenized along the normalized radius during normal loading. On the other hand, the experimental studies of (O'Connell et al. 2007), (O'Connell et al. 2011) and (Tsantrizos et al. 2005) found that the AF, regardless of the region, is in a radial tensile state when the entire IVD is loaded under compression, flexion or lateral bending. Moreover, the numerical study of (Yang et al. 2018) shows large radial strains due to the swelling properties developed by the high glycosaminoglycan content. Therefore, the radial residual strains would tend to decrease the global radial strain state generated by both mechanical and chemical loads. In regards to all these features, it would be interesting to integrate an initial residual strain field in a numerical study by using the multiplicative decomposition of the deformation gradient, as proposed by (Jha et al. 2019; Pena et al. 2006; Rausch et al. 2013; Balzani et al. 2006), and to compute its influence on the mechanical and nutrient transport fields. To this end, we propose a simple *cosinus* sum function model based on normalized radius and angle to fully describe the residual strain state within the IVD. Finally, in addition to the homogenization of the *in vivo* strains and stresses of the AF, the residual strains could also contribute to increase the global stiffness of the vertebral column.

The origins of such residual strain fields are still a matter of debate, and it is unclear whether these come from a gradient in glycosaminoglycan or a gradient in growth? While the circumferential strains that we see in the circumferential direction are equivalent to those induced by the osmotic pressure in the study of (Yang et al. 2018), our analyses of residual strains in the radial direction lead to completely different results. These large discrepancies highlight the fact that residual strains may not arise only from the osmotic pressure but also from other phenomenon such as growth and remodeling. However, it seems difficult to segregate the impact of osmotic pressure from the growth and remodeling process. Indeed, during the growth and remodeling process both collagen proteins and glycosaminoglycan are secreted. While the new created matter will exert forces on the old matter, the new glycosaminoglycan will increase the negative charge density of the AF and thus increase the osmotic pressure. One possible way to determine the impact of glycosaminoglycan distribution would be to realize residual strain relaxation in hypertonic and hypotonic conditions as has already been done on arteries by (Azeloglu et al. 2008). We have carried out some preliminary experiments by modifying the NaCl bath from an isotonic saline (9g/L) to a hypertonic saline (100g/L). We observed contraction by less of 2% in the radial direction and dilatation by up to 2% in the circumferential direction. These preliminary results suggest that the effect of negative charges is of minor importance in residual strain genesis but further studies are needed to fully understand this phenomenon. It is now of crucial importance to develop growth models that take into account the multiplicative decomposition of the gradient of the deformation but also the creation of new negative charge (Armstrong et al. 2016). Finally, the strong linear correlation between the level of residual strains at the inner periphery and the gradient of these same strains along the normalized radius (fig 8) highlights the spatial interdependence of the residual strains. Such a phenomenon could be key point to the understanding of growth pattern and the development of new IVD growth laws.

One major limitation of our work is the impossibility to determine residual strains in the axial direction. To this end, it may be possible to develop an experimental setup with a stereo-correlation technique. It could also be relevant to develop an adapted white and black powder to optimize the speckle pattern and thus greatly refine the digital image correlation results. The use of such a powder could also allow a more detailed study of the tensile test sample as well as the analysis of failure mechanisms of the AF. In addition, better experimental conditions would allow us to minimize the use of the pointwise least square technique to smooth the results. Indeed, a sensitivity study shows us that we probably underestimate the circumferential and radial strains in the lateral area because of our circular window of 40 pixels (5% to 30% of the radial thickness of the samples). The objective was to find a balance between softening the noisy experimental results and accounting for the large values of residual strains. Finally, as pointed out by (Lee et al. 2020), most of the studies on residual strains focus on the continuous scale whereas the microscopic scale seems essential. Therefore, future studies should combine the relaxation of residual strains by the radial incision technique and the microscopic study of the *annulus fibrosus*.

5 Conclusion

By combining different methods, namely radial incision and digital image correlation, this study highlights the complex residual strain fields in the *annulus fibrosus*. In particular, we identify a strong and zone dependent circumferential residual strain gradient along the normalized radius, as well as stable and strong

radial compressive strains. Our results show that our DIC technique is a robust tool for the characterization of residual strains on the *annulus fibrosus* and highlights its potential for application to human samples. Our study shows that numerical models of the AF should integrate residual strains to better mimic the *in vivo* behavior of the IVD. We hope that this work will lead to a better understanding of the origin of residual strains and allow a more detailed characterization. Future work could focus on assessing the impact of the anatomical level on the pattern and intensity of residual strains.

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