Review of “Residual strains estimation in the annulus fibrosus through digital image correlation”

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Review of "Residual strains estimation in the *annulus fibrosus* through digital image correlation"

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1 Review of version 1

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1.1 Reviewer #1 (Anonymous)

Summary

Reviewer This manuscript details an experimental study aimed at mapping residual strains in the porcine intervertebral disc annulus fibrosus. While similar measurements have been made by other groups using caudal discs, the present work is the first to expand this idea to discs with a more complicated geometry. While the methodology appears to be mostly sound, there are some concerns (detailed below) about both the sample size and possible artifacts in the analysis procedure.

Authors We thank the reviewer for their helpful comments.

General comments

Reviewer The manuscript contains numerous typos and unclear sentences. A thorough proofreading is required.

Authors A thorough proofreading has been done.

Reviewer The study utilized ten IVDs, which were all from the same spine. Strain measurements from all of these discs were averaged for analysis. There are significant variations in both geometry and mechanical loading throughout the porcine spine. Recent work by Duclos et al (J Exp Biol, 2020) suggests that residual strains may vary with mechanical load amplitudes even if geometry does not vary. If it is not possible to add additional spines to the sample group, it may at least be helpful to separate the ten discs measured here into anatomical groups (cervical, thoracic, lumbar) for comparison.

Authors Thank you for the relevant comment, we compared the gradients and offsets of residual strains between the different anatomical groups but we did not notice any apparent difference. However, given the small sample size, a new study could be conducted to identify differences between anatomical groups. A sentence has been added to the conclusion of our article: "*Future work could focus on assessing the impact of the anatomical level on the pattern and intensity of residual strains.*"

Specific comments

Reviewer Title: "Enhanced" suggests that digital image correlation is producing a better measure of residual strains in the porcine AF, though these measurements are the first.

Authors The article title has been changed to: residual strains estimation in the pig (?) annulus fibrosus through digital image correlation.

Reviewer Introduction: The authors state that the opening angle experiment has only been performed by Michalek et al (2012) and Duclos et al (2017). This analysis has also been performed by Mengoni et al (R Soc Open Sci, 2017). Also, the authors rightfully point out that the Duclos 2017 arc length approach assumed cylindricality, however, circumferential strain was also measured by fiber crimp period in that paper.

Authors Indeed, Mengoni et al (R Soc Open Sci, 2017) also performed a residual strains analysis on bovine caudal tissue. They also used the opening angle method to estimate the circumferential residual strains. The work of Mengoni et al (R Soc Open Sci, 2017) is now cited in the introduction: "*To this date, only the work of (Michalek et al. 2012), (Duclos et al. 2017) and (Mengoni et al. 2017) have intended to estimate 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 lamellae thickness evolution during the stress relaxation experiment. However, the global residual strains estimation of these studies is based on the roundness of the tissue, which make it difficult to reproduce on the more complex geometry of human AF. Noteworthy, (Duclos et al. 2017) also used the polarization birefringence microscopy to estimate the fiber crimped period before and after the radial incision. This method shows residual strains qualitatively equivalent to the arc length method but quantitatively different.*"

Reviewer Section 2.1: Please specify exactly which anatomical levels of the spine were used.

Authors The anatomical levels of the spine have been specified: "*A total of ten annulus fibrosus sheets specimens were harvested from one large white pig spine of three-month-old (≈ 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) AF have been extracted.*"

Method

Reviewer Please include a description of the image acquisition procedure including instrumentation. Additionally, please indicate the amount of time that elapsed between radial cutting of the AF and imaging. One concern with using such a thin specimen is that if immersed in saline, it will swell rapidly. This can lead to an over-estimate of residual strains, particularly in the inner AF.

Authors Thank you for your comment. A description has been added at the end of the first paragraph of the method section: "*The samples were then immersed in a physiological solution of NaCl (*9 g/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* 0.1 Hz *for 4 hours during the residual strains relaxation period. The entire experimental set-up is shown in Figure 1.*"

An illustration has also been made to visualise the whole measurement system, see Fig. 2 in the last version of the article.

For preservation, the spine was frozen in physiological NaCl solution (9 g/L). On the day of the experiment, it takes 10-15 minutes to extract the AF from the adjacent vertebral endplates. Once extracted, the AF is placed directly into the saline solution for imaging. Coming from and going to a physiological solution of 9 g/L NaCl, there is no variation in osmotic pressure. Therefore, the disc is not supposed to swell due to the physical phenomenon of osmotic pressure. We therefore added that the spine was frozen via a physiological NaCl solution (9 g/L) in the first paragraph of the method section: "*The spine has been obtained from the local laboratory animal house and immediately frozen at* −20 °C *in a physiological solution of NaCl (*9 g/L*).*"

Reviewer Equation numbering starts with the second equation. Additionally, the last line of page 2 includes "(see eq. ??)".

Authors The equation numbering now starts with the first equation. The line with reference issue "see eq. ??" has been corrected: "*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.*”

Reviewer Section 2.3.1: Please include physical size (mm) along with image size (px) for correlation windows. Addition of a scale bar to Figure 1 would also be helpful for contextualizing these numbers.

Authors Physical size in mm has been added to section 2.3.1: “*For each point, a subset dimension of* 85 px × 85 px *(*4.6 mm^2*) was searched in a* 150 px × 150 px *(*8.1 mm^2*) research area to identify both the displacement and the rotation of the subset.*” The scale bar and correlation window size are now present in figure 1.

Reviewer Section 2.3.3: Pointwise least squares was performed across an arbitrary 40 px window size. How does 40 pixels relate to the thickness of the AF? Was a sensitivity analysis performed to justify this window size?

Authors This is a very good point. Depending on the disc studied, a window size of 40 pixels represents 5 to 30 % of the radial thickness of the AF. Following your question, we conducted a sensitivity study to justify the choice of window size. We noticed that on the whole AF, the gradients and offsets of residual strains are not very sensitive, on average, to the window size (circumferential: gradient amplitude: 0.1 to 0.8, offset amplitude: 0.04 to 0.016 , radial: gradient between 0.01 and 0 and offset in 0.65 and 0.035), see figures 1 and 2. Except for the offset of the radial strains which

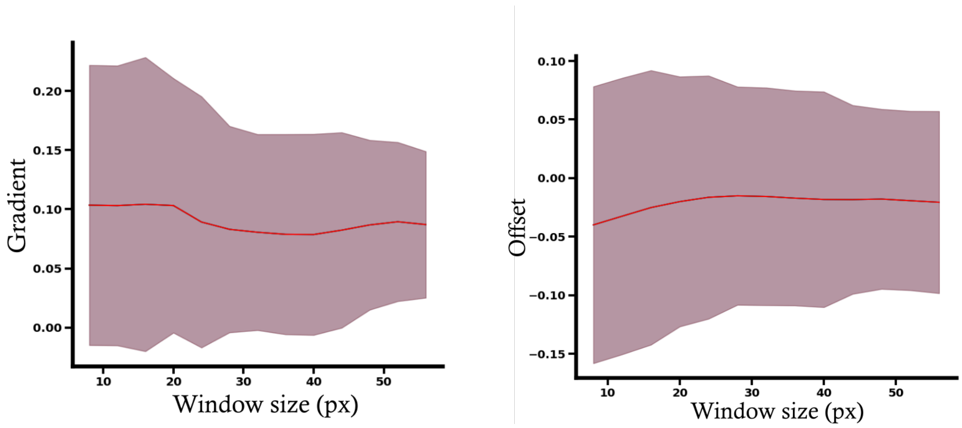

Figure 1 Convergence of the gradient and offset of circumferential strains as a function of window size throughout the AF on average.

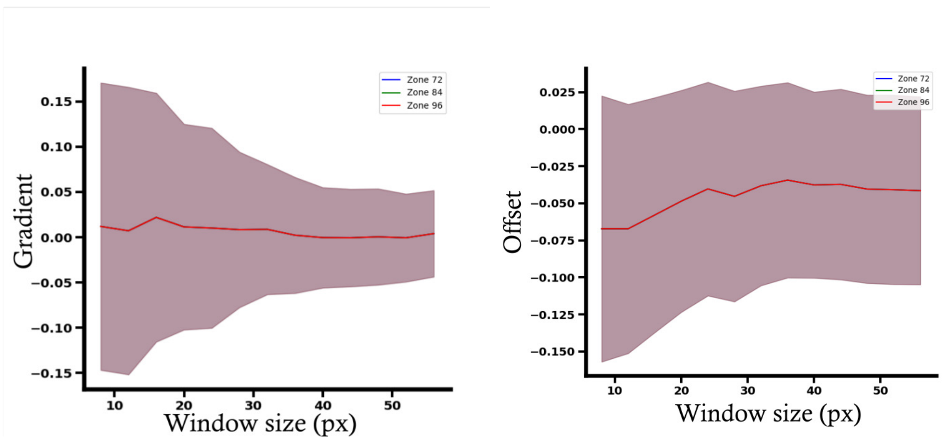

Figure 2 Convergence of the gradient and offset of radial strains as a function of window size across the AF on average.

goes from very low values (−7 %) to less low values (−4.5 %). This means that the absolute values of the radial strains are sensitive to the size of the windows but not their evolution along the normalized radius.

When performing the sensitivity study, we noticed that the lateral areas (angular portion of 72°, 84° and 96°) are the most sensitive. We believe this is because the radius of curvature in this area is very small. Therefore, if a window size is too large, the results in the lateral zone are affected by the measurements in the anterior and posterior zones. Our sensitivity study investigated the change in the gradient and offset of the residual strains as a function of the window size.

The sensitivity study gives us the following results on the gradient and offset of the circumferential and radial residual strains, see figures 3 and 4. Here again, it can be seen that the

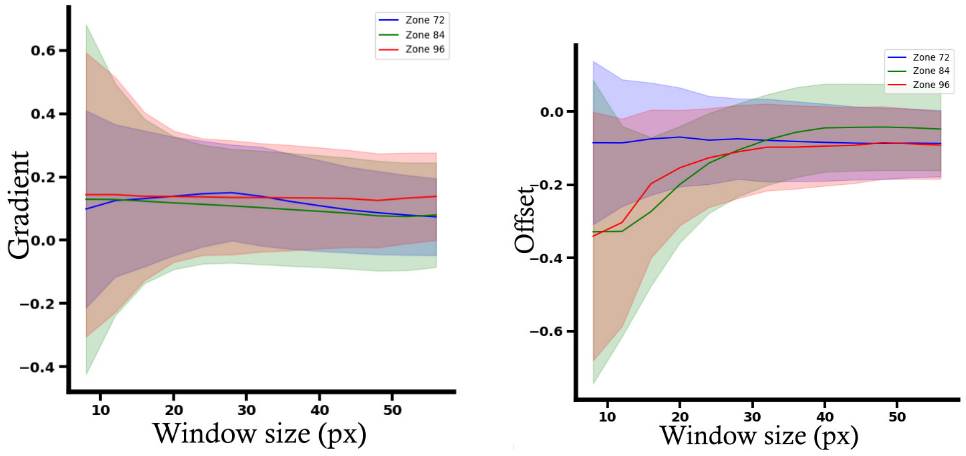

Figure 3 Convergence of the gradient and offset of circumferential deformations as a function of window size in the "sensitive" areas.

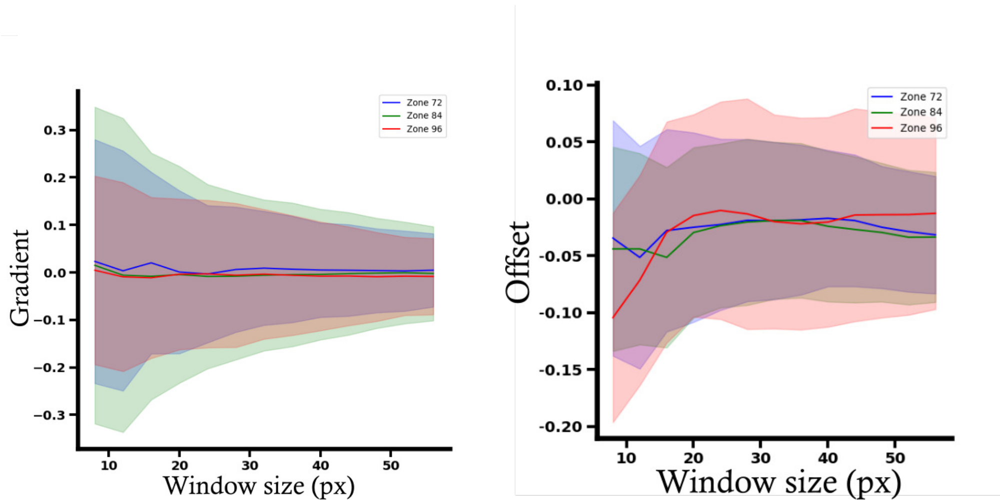

Figure 4 Convergence of the gradient and offset of radial deformations as a function of window size in the "sensitive" areas.

gradient of the residual deformations is not affected by the size of the windows. Only the offset is sensitive to this dimension, especially in the circumferential direction. This means that the evolution of the residual strains along the normalized radius remains identical whatever the window size, but the absolute values are modified. In view of the difficulty in choosing a window size, it seems more appropriate to discuss this parameter in the discussion section.

As a result, we have added a sentence in the discussion section to "put our results into perspective", especially in the sidebar. The sentence is as follows: "*In addition, better experimental conditions would allow us to minimise the use of the pointwise least square technique to smooth the results. Indeed, a sensitivity study has shown us that we probably underestimate the circumferential and radial strains in the lateral area due to our circular window of 40 pixels (*5 % *to* 30 % *of the radial thickness of the samples).*"

Reviewer Section 2.5: What was the justification for assuming a linear trend in strain with radial position? This was not the case in Fung's blood vessel work.

Authors The linearity assumption is made locally on the dimension of the least squares window (i.e. on sizes of 2.2 mm × 2.2 mm). In spite of this local assumption, we notice that the evolution of the residual deformations along the radius can be non linear. This is for example the case for the radial residual deformations.

Furthermore, for the circumferential deformations, it can be seen that the deviation from the linearity assumption is small for the circumferential deformations (NRMSE = 12.54 %) and slightly higher for the radial and shear deformations (respectively NRMSE = 20.9 % and 21.2 %).

Reviewer Statistics: The Kruskal-Wallis test seems inappropriate here, as it does not account for the order of sampling. As the measured strains are discrete samples of things that are assumed to vary continuously with angle, a regression analysis may be more appropriate.

Authors The Kruskal Wallis test (non parametric ANOVA) remains valid to show that on a batch of intervertebral disc, we notice a dependence of the residual strains according to the radius and according to the angle. We made this choice to follow the spirit of the work of Duclos and Michalek in 2017 in the article: "Residual strains in the intervertebral disc annulus fibrosus suggest complex tissue remodeling in response to in vivo loading". We chose to use a non parametric test because of the non normality of the distribution of the results.

Reviewer Figure 5(a): The average circumferential strain field is less than zero throughout the entire thickness of the AF on the radial sides, which violates static equilibrium for any non-zero elastic modulus. This is likely the result of the significant edge effects seen in Figure 3.

Authors The annulus fibrosus has just been removed from the adjacent vertebral endplates and the nucleus pulposus has also been removed. As the annulus fibrosus is filled with residual deformations, it is normal that the static equilibrium is not respected when the adjacent organs are no longer linked to ensure this equilibrium, and this just after the extraction of the disc and before the relaxation of the internal constraints. At the end of the relaxation phase, we find a state of equilibrium of the AF that is not constrained by the natural attachments it had before extraction. The starting point for our reconstruction of the residual strains is this equilibrium point, after relaxation of the internal stresses (open configuration). Obviously, when we re-impose the reverse kinematics of the disc opening, we put the AF back into a situation where reaction forces at the AF's attachment nodes on the natural attachment zones are necessary to make it regain its physiological shape. The existence of these reaction forces at the natural anchor points of the AF is necessary to understand the fact that, for example, the average circumferential stress is probably not zero.

Moreover, one should not forget that the mechanical properties of FA are not homogeneous depending on the zone and the normalized radius (Dusfour et al., 2020, "Heterogeneous mechanical hyperelastic behavior in the porcine annulus fibrosus explained by fiber orientation: An experimental and numerical approach." Journal of the Mechanical Behavior of Biomedical Materials, 104), which implies that the average of the circumferential strains may be non-zero while the average of the circumferential stresses may be zero. In fact, the average of the circumferential stresses does not seem to be zero either (calculation not shown) and the first remark concerning strong reaction forces at the natural grip zones of the disc when it has its physiological shape will allow us to understand that our results do not call into question the principle of static equilibrium.

Reviewer Table 1 suggests that the cosine series in Equations 9 and 10 were carried out to $n = 3$ and $n = 2$, respectively. However, Figure 5 has some artifacts in the anterior AF that look like they are at a higher spatial frequency. Please clarify.

Authors Yes, the cosine series was performed only with $n = 3$ and $n = 2$ to limit the number of parameters while fitting the experimental data properly (gradient: 14 % error and offset: 12.85 % error). We could add more parameters but this would make the model more cumbersome. Our wish is to show that the evolution of the gradient and the offset along the angle can be modelled with a relatively simple continuous function, without increasing the number of coefficients.

Reviewer Figure 5(a,c): Duclos et al (2017) showed anti-correlation between radial and circumferential strains. In the present work, there are locations where the two components are either both positive or both negative, pointing towards a zero or negative Poisson's ratio.

Authors We thank reviewer 1 for this precise comment. Indeed, there are locations where the two components of strains are both positive or both negative, especially in the anterior area. This feature highlights that AF can have negative Poisson's ratio. In addition, we have already highlighted such a characteristic in our previous work (Dusfour et al 2020), in which traction experiments were carried out on the same samples as this study. Therefore, the third paragraph in the discussion section has been modified to highlight such a characteristic: "*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 that AF can locally have negative Poisson's Ratio, as already shown by (Dusfour et al. 2020).*"

Reviewer Figures 4 and 7: Please indicate what the closed black circles represent.

Authors In Figure 4 (now Figure 5), the black circles represent the outliers of the boxplot. A sentence has been added to the description in figure 5: "*Closed black circles represent boxplot outliers.*"

In Figure 7 (now Figure 8), each black circle represents a portion of the fifteen angular portions of each of the discs. In each of the angular portions (angular amplitude of 12°) we have recovered the residual deformation gradient along the normalised radius as well as the average value of the residual deformations on the inner periphery (offset). These figures show that the residual deformations on the entire disc can be predicted only by measuring the deformations on the inner periphery. A new sentence has been added to the figure: "*These figures show the linear relationship between the strains 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.*"

2 Review of version 2

Permalink: hal-03058316v2

2.1 Reviewer #1 (Anonymous)

Reviewer The authors assert, without evidence, that four hour immersion in 9 g/L saline was not supposed to result in tissue swelling. The effect of immersion in saline on the swelling behavior of IVD tissue has been well-documented, and is the reason why prior measurements of residual strain used images acquired immediately after strain relief. What is being described here as residual strain relaxation is almost certainly the result of free swelling. Swelling of the tissue is the most likely explanation for the authors' observance of both a negative Poisson's Ratio and residual compression throughout the entire thickness of the AF.

Authors The concentration of 9 g/L corresponds to a physiological value that we respect in our study. A variation around 9 g/L would have an impact on the deformation state of the tissue. We chose to place ourselves in physiological conditions to highlight the residual deformations of the tissue in vivo all as in Michalek et al. 2012. We are aware that the residual deformations that we highlight are the result of different phenomena (swelling, growth, remodelling and other unknown phenomena) as indicated in the second last paragraph of our discussion. We also choose to wait for the complete relaxation of the visco/poro elasticity phenomena of the tissues to obtain the state closest to the mechanical equilibrium.

We have modified the second last paragraph of the discussions as follows: "*These large discrepancies highlight the fact that residual strains* do not *may not arise only from the osmotic pressure but also from other phenomenon such as growth and remodeling.*"

- Michalek, A., Gardner-Morse, M., and Iatridis, J. (2012). Large residual strains are present in the intervertebral disc annulus fibrosus in the unloaded state. Journal of Biomechanics, 45(7):1227-1231.

2.2 Reviewer #2 (Anonymous)

Reviewer The paper deals with a topic that is attracting a lot of attention lately since it focuses on the stability of blood vessels. Clearly there is a lot to do to understand the mechanisms at hand. Axial pre-stretch has been the main focus modeling soft tissue but clearly azimuthal pre-strain plays a mayor role in vessel instabilities. Residual stress has been analyzed to understand aneurysms formation. Recent papers considered the pre-stress modelled using the opening-angle method. I refer the authors to the analysis in these papers that establish a residual stress tensor and the authors may establish some connections with their work:

- Merodio J. and Ogden R.W. (2016) Extension, inflation and torsion of a residually stressed circular cylindrical tube. Continuum Mechanics and Thermodynamics 28(1-2):157-174.
- Dehghani H., Desena-Galarza D., Jha N.K., Reinoso J., Merodio J. (2019). Bifurcation and post-bifurcation of an inflated and extended residually-stressed circular cylindrical tube with application to aneurysms initiation and propagation in arterial wall tissue. Finite Elements in Analysis and Design 161:51-60.
- Jha N.K., Reinoso J., Dehghani H., Merodio J. (2019) A computational model for fiber-reinforced composites: hyperelastic constitutive formulation including residual stresses and damage. Computational Mechanics 63(5):931-948.

Stress and strain finally have to be linked through a constitutive modeling.

Authors 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. We have added the following quotation from the article, at the end of paragraph 4 of the discussion, which seems relevant to our study for the integration of residual strains in numerical models:

- Dehghani H., Desena-Galarza D., Jha N.K., Reinoso J., Merodio J. (2019). Bifurcation and post-bifurcation of an inflated and extended residually-stressed circular cylindrical tube with application to aneurysms initiation and propagation in arterial wall tissue. Finite Elements in Analysis and Design 161:51-60.

3 Editor's assessment (A. Pandolfi)

The revision of this paper has been difficult. After many denials, a competent reviewer accepted to read and comment on the manuscript. The review was accurate, and generally positive, and asked for a major revision. Among the questions, it was mentioned the difficulty of estimating the residual strains in the annulus fibrosus. The response to the reviewers by the authors was accurate and provided answers to all the points, leading to important changes in the manuscript.

Surprisingly, the second review of the second version of the manuscript by the same reviewer was totally negative, rating the manuscript with 0 in all points and asking for a rejection, because the first review comments were not answered. The review was accompanied by a unique negative comment on a possible misinterpretation of experimental free swelling as strain relaxation. Regrettably, this comment was not explicitly included in the first review, and the rejection was, in my opinion, unmotivated and unfair.

A second reviewer was then invited for the review of the second version of the manuscript. The second reviewer found the work acceptable with minor revision.