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DIGITAL IMAGE CORRELATION ON ANNULUS FIBROSUS SOFT TISSUE

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ABSTRACT: To characterize the mechanical behaviour of soft biological tissue, various experimental constraints appear. Actually, physiological conditions are generally required to preserve material properties. Thus, samples must be immersed in a thermo-regulated bath during experiments which prevent from using classical strain measurement techniques. The alternative is to rely on image analysis methods which present the benefit to be non-invasive.

This work focuses on annulus fibrosus tissue (AF), i.e., the external tissue of intervertebral disc located between consecutive vertebral bodies. Lumbar discs were harvested from cadaver of a domestic pig and prepared to perform a tensile test in physiological conditions. Two high resolution cameras are positioned perpendicularly to the tensile direction to visualize both transverse planes. By using Digital Image Correlation technique on natural pattern, surface displacement and strain fields are obtained. Heterogeneous fields are observed in all directions with strong strain localisations. Transverse strain in the plane of lamellae highlights the porous nature of AF tissue with a swelling in the central part and symmetric strain gradients directed towards both sample sides. Over multiple tensile loading cycles, some hysteretic loops are observed on both stress and strain evolutions emphasizing the essential dissipative function of such material. A poro-mechanical explanation associated with the biphasic and composite constitution of AF tissue is proposed allowing to quantify coupling effects between the mechanical behaviour and liquid flows.

This method lead to develop a better identification procedure by fitting numerical models with 3D tissue surface behaviour. Introducing such description of the poro-mechanical behaviour of annulus tissue will have strong implications on the coupling between strains and fluid transfers.

1. INTRODUCTION

To characterize the mechanical behaviour of soft biological tissue, various experimental constraints appear. Actually, physiological conditions are generally required to preserve material properties. Thus, samples must be immersed in a thermo-regulated bath during experiments which prevent from using classical strain measurement techniques. The alternative is to rely on image analysis methods which present the benefit to be non-invasive.

This work focuses on annulus fibrosus tissue (AF), i.e., the external tissue of intervertebral disc located between consecutive vertebral bodies. When vertical loads are transmitted along the spine, its role is to hold the over-pressure created in the central part of disc [1]. In this configuration, annulus fibrosus is mainly subjected to circumferential tensile stresses. Owing to its particular microstructure, i.e, woven oriented fibres embedded in a highly hydrated matrix (60 – 70%), AF owns a non linear and anisotropic behaviour. Furthermore, this porous tissue is saturated by physiological liquid leading to coupling effects between the mechanical behaviour and liquid flows. To analyse the annulus fibrosus physiological behaviour, these coupling effects must be taken into account.

From a mechanical point of view, it is considered as a natural composite material with oriented fibres which gives an anisotropic and non linear mechanical behaviour [2, 3, 4, 5]. Stress non-linearities are attributed to progressive tightness of collagen fibres and to the reorientation of fibres along the mechanical load [6, 5]. As a porous tissue, strain rate plays an important role on bi-phasic AF behaviour and a lot of works use slow strain rate, $\dot{\epsilon} \approx 0.0001 \text{ s}^{-1}$, to avoid transient effects due to fluid flows [2, 3, 4]. Larger strain rates, $\dot{\epsilon} = 0.001 \text{ s}^{-1}$, are used in this work to precisely emphasize and characterize poro-mechanical coupling effects.

To compute displacement measurement, image analysis methods need a contrasted pattern to follow some markers along mechanical test. In literature, we mainly find glued markers [4, 6, 7], pins inserted through tissue thickness [3] and stained tissue by using laser or chemical baths [8]. Nevertheless, image analysis are based on surface displacement, thus physical makers and glue on soft tissue can disturb surface behaviour and impact strain measures even if considered negligible. Less invasive works used grey scale thresholding [9] or cell nucleus to investigate micro-structural strains [4]. In all cases, the number of markers does not exceed 100 [2].

Therefore, this contribution aims at investigating the transverse behaviour of annulus tissue samples subjected to uni-axial tensile tests. Digital image correlation (DIC) technique allows to determine the transverse strain fields while the sample is immersed in a thermo-regulated physiological solution. To prevent from any measurement perturbation, the natural AF pattern is used. Results clearly highlight the anisotropic feature of annulus tissue. By comparing the strains obtained in both transverse directions, some coupling effects associated with fluid transfer are underlined. This means that the annulus fibrosus is essentially a porous material, justifying the integration of fluid flow characteristics when modelling its mechanical behaviour.

2. MATERIALS AND METHODS

Lumbar discs were harvested from cadaver of a domestic pig obtained in a local slaughter house. They were separated from the vertebral bodies by blunt dissection. Then, posterior, lateral and anterior specimens were excised in anatomical cut, parallel to the horizontal plane along the circumferential direction \vec{e}_θ . The circumferential length of each specimen, $L_{\theta\theta}$, was limited using a surgical knife at approximately 10 mm. Then, to get stuck into the testing device grips, both specimen ends were glued on aluminium cylinders using cyano-acrylate adhesive (Fig. 1) [5, 9]. Finally, 19 plane-parallel porcine samples ($L_{rr} \times L_{\theta\theta} \times L_{zz} = 3.88 \pm 0.99 \times 10.40 \pm 2.48 \times 4.13 \pm 0.93 \text{ mm}^3$) were prepared to analyse the transverse behaviour of annulus tissue.

Mechanical tests are realized *ex situ* with *in vivo* conditions, 0.15 mol.l⁻¹ NaCl solution at $T = 37^\circ\text{C}$. The testing device is composed of a texture analyser (LF-Plus, Lloyd Instruments) equipped with a 50 N load cell. Two high resolution cameras (AVT stingray, 16 bits, 1600 × 1200 pixels) are positioned perpendicularly to the tensile direction, \vec{e}_θ , to visualize both transverse planes through a transparent bath filled with a controlled physiological solution (Fig. 1). Prime lenses are set on cameras leading to a spatial resolution of 3.65 μm/pix.

The objective is to perform uniaxial tensile tests. To respect physiological solicitations, we imposed a longitudinal strain of $E_{max} = 10\%$ which has been reported to be the maximum observed strain during physiological motions [1]. The experimental procedure relies on three steps. First of all, a conditioning test of 10 loading cycles is performed on each sample to break the fibres damaged during the sample preparation and control the sample ends gluing quality. Then, the sample is retained in the bath at zero strain during one hour so as to reach a poro-mechanical equilibrium. Finally, the tensile test is performed and digital images are shot every 4 s in both transverse directions, \vec{e}_z and \vec{e}_r . A displacement of 1 mm was imposed at a velocity of 0.01 mm.s⁻¹ which corresponds to a strain rate of 10⁻³ s⁻¹.

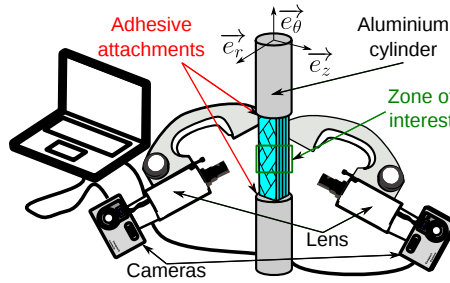


Figure 1 - Tensile test principle

The images analysis procedure is based on a Digital Image Correlation method. An academical software (*KelKins*) is used, a detailed description of the method can be found in [10]. It provides the local displacement field in each point of an initial chosen Cartesian grid. Digital images are focused in the central part of samples to increase pixel accuracy and avoid side effects resulting from glue bonds on aluminium grips. A grid of 20 × 28 points is linked to this region which leads to a measurement point every 0.18 mm. In some cases where the sample sizes are lower, this grid can be reduced until 14 × 20 points.

When using such DIC methods, samples are generally artificially speckled by using black paint projected on a white surface, or *vice versa*. This leads to highly contrasted images of randomly disposed pattern. Such preparation is inconceivable with annulus samples immersed in a physiological bath and correlation computations must rely on the contrasted nature of tissue. The moving windows has been widened to 0.18 × 0.18 mm² in order to get better and more discriminating correlation indicators. Resulting from the complex anisotropic micro-structure of annulus tissue, the displacement fields observed are significantly heterogeneous without preferred spatial direction. Therefore, the research windows cannot be constrained using the displacement imposed at both sample ends and requires to be increased up to 0.36 × 0.36 mm². The consequence is a higher computational cost but leads to accurate displacement measurements.

Since the optical path crosses 4 different environments (lens, air, glass, solution), this induces optical distortions and measurement discrepancies. Furthermore, due to the large strains observed locally (see next section), keeping the initial image at zero strain as the reference image for correlation computation do not ensure a systematic DIC algorithm convergence. The alternative is to update the reference image at each time step. However, the measurement errors are cumulated over the whole experiment, i.e., over the 25 images shot during the tensile test.

Strain discrepancies are estimated in testing conditions with the thermo-regulated bath using one of the annulus samples. The principle is to create a rigid body motion by imposing a known longitudinal displacement to the sample and compare it to the displacement measured in each point by the DIC procedure. Using a similar approach, strain measurement errors due to out of plane displacement have been estimated. In all cases, they do not exceed 0.1% on average.

These various optical distortions generate measurement errors fairly described by a randomly distributed noise. Therefore, a filtering process is applied. It is based on a Savitsky-Golay algorithm using a quadratic polynomial surface and a centred moving windows including 25 neighbouring points. This smoothing procedure leads to remove two rows of point along the side of the region of interest. This has the advantage of eliminating the measurement points where DIC boundary effects are concentrated leading to unjustified strain concentrations. Finally, the number of measurement points reduces from 463 to 305 points.

3. RESULTS

This work focuses on the transverse mechanical behaviour in both directions, radial \vec{e}_r and vertical \vec{e}_z . The plan of fibres is given by circumferential and vertical directions, $(\vec{e}_\theta, \vec{e}_z)$, while the plan of lamellae refers to the circumferential and radial directions, $(\vec{e}_\theta, \vec{e}_r)$. Using the images analysis procedure detailed in previous section, bi-dimensional strain fields are identified in both planes. One example of longitudinal strain fields, i.e. in the circumferential direction \vec{e}_θ , are given in Fig. 2 at the end of tensile test. The strain fields computed from DIC displacement measurements are superimposed over the sample image. The white grid gives an indication of the overall deformation while the grey scale represents the longitudinal normal strain, $E_{\theta\theta}$.

One can note that longitudinal strain fields are significantly heterogeneous and do not show any clear tendency. Strain local values range from 0% to 20% even though the macroscopic imposes strain is about 10%. It means that the average value is consistent while strong localization effects takes place. Similar conclusions could be drawn with the whole set of experimental tests. In some cases, local longitudinal strain goes up to 30%. These localization effects should be associated with the complex and anisotropic micro-structure of annulus tissue.

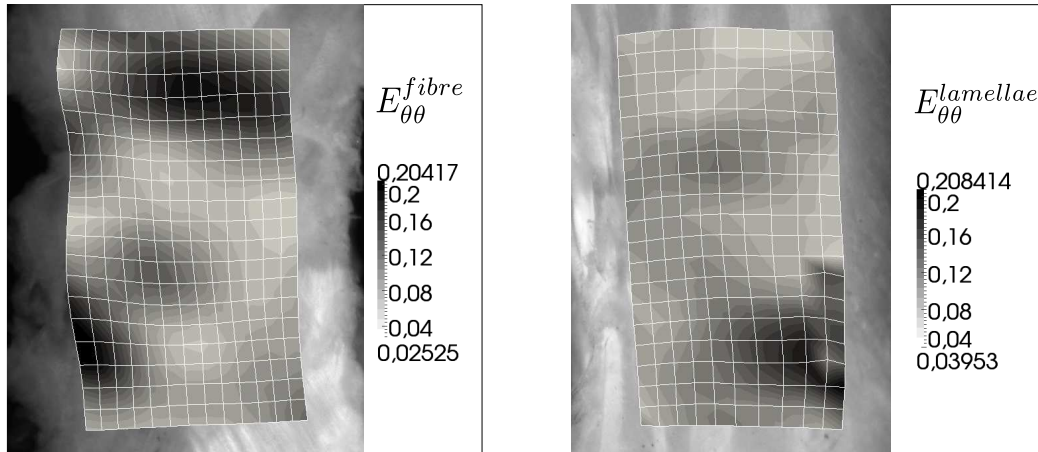


Figure 2 - Circumferential normal strain fields in the fibres plane and the lamellae plane

Regarding the same experimental test, transverse behaviours in both planes, i.e., vertical normal strain, E_{zz} , in the plane of fibres and radial normal strain, E_{rr} , in the plane of lamellae, are given in Fig. 3. In the fibres plane, strain field shows an heterogeneous shrinkage with an amplitude similar to the one observed in the circumferential direction (Fig. 2). The heterogeneity pattern of this strain field looks like the one obtained in circumferential direction and, once more, seems to reveal the underlying micro-structure. The description of such effects is not addressed here since the objective is to analyse the macroscopic homogenized behaviour of annulus tissue.

Unlike previous strain fields, the transverse behaviour in the plane of lamellae points out some macroscopic features that are not associated with the micro-structure. The strain field given in Fig. 3 clearly reveal vertical strips with a strong swelling in the central part decreasing until shrinkage on sample sides. Transverse strains up to 42% can be observed locally. The transverse strain gradients are more or less symmetric with respect to the sample longitudinal axis.

Considering the 19 samples under investigation, 11 present such a behaviour. The average value could be really different from one test to the other, nevertheless the overall shape of radial strain fields show similar features, i.e., a central part with a larger swelling or lower shrinkage and symmetric decreasing strain gradients towards both sides of sample.

4. DISCUSSION

Uniaxial tensile test is the classical experimental technique to characterize the mechanical behaviour of biological tissue. When dealing with complex anisotropic material such as biological tissue, the stress/strain curve does not bring out sufficient data to identify material properties. Results can be easily enhanced by analysing the transverse behaviour. The opportunity to rely on images analysis method allows many difficulties to be overcome, especially associated with the short size of samples and the immersion in a physiological solution. In this framework, Digital Image Correlation techniques allow to extract a large amount of data (about 300 measurement points in our case) from standard images and provide bidimensional strain fields. For instance, one of the main discrepancies results from the irregular geometrical boundaries since conventional tissue carving hardly leads to plane-parallel samples. The large number of measurement points allows to focus on the central area of samples and avoid side effects.

In spite of the weakly contrasted natural pattern of annulus tissue, the convergence of correlation algorithms is accurate. Taking into account the optical distortions resulting from the variety of media crossed by the optical path (lens, air, glass, water), DIC errors have been estimated to be about 0.1% in average without exceeding 0.8% locally. When compared to the 10% longitudinal strain imposed and to the $[-22\%, 42\%]$ strain range measured, DIC discrepancies cannot significantly influence the results or hide partial informations. However, the large strains observed confirms that the use of hyper-elastic

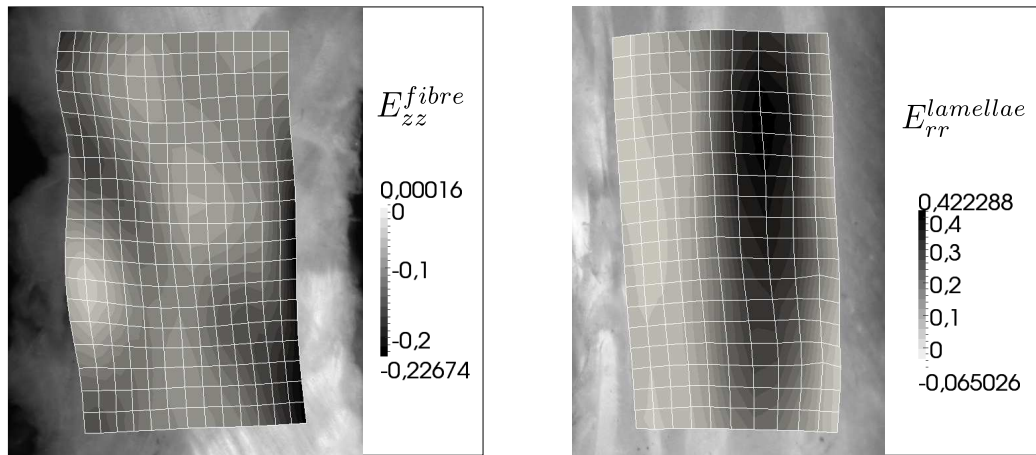


Figure 3 - Vertical and radial normal strain field at maximal load

models is required to describe the AF mechanical behaviour.

At the millimetric scale, strain fields underline the heterogeneous tissue behaviour. Actually, in circumferential direction, amplitude strain are twice than the imposed with strong heterogeneity. Transverse strain field in lamellae plane, Fig. 3, shows a central strip with positive strains indicating a swelling. This anisotropic behaviour and large swelling strains leads us to think that fibres reorientation is not the only governing phenomena [5]. Since it is saturated by a solution, the coupling between mechanical behaviour and fluid transfers plays a prevailing role. Its particular coupled behaviour directly results from the anisotropic stiffnesses. Indeed, the woven collagen structure provides high rigidities in the plan of fibres while the elastin matrix leads to low rigidities in the direction of lamellae. A porous explanation could be that the strong transverse shrinkage, in the plane of fibres, generates a fluid over-pressure inside the porous matrix that discharges in the perpendicular transverse radial direction.

Using plane displacement fields given by DIC leads to better identification procedures by accounting for 3D surface behaviour. Introducing such description of the poro-mechanical behaviour of annulus tissue will have strong implications on the coupling between strains and fluid transfers [11].

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