Characterization of chemoelastic effects in arteries using digital volume correlation and optical coherence tomography.

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Abstract
Understanding stress-strain relationships in arteries is important for fundamental investigations in mechanobiology. Here we demonstrate the essential role of chemoelasticity in determining the mechanical properties of arterial tissues. Uniaxial tensile tests were carried out on samples of porcine aortas immersed in a hyperosmotic solution. The tissue deformations were tracked using optical coherence tomography (OCT) during the tensile tests and digital volume correlation (DVC) was used to obtain measurements of depth-resolved strains across the whole thickness of the tested aortas. The hyperosmotic solution exacerbated chemoelastic effects, and we were able to measure different manifestations of these chemoelastic effects: existence of an osmotic modulus on top of the elastic modulus, swelling of the media inducing a modification of its optical properties, existence of a transverse tensile strain (negative Poisson’s ratio). For the first time ever to our best knowledge, 3D strains induced by these chemoelastic effects in soft tissues were quantified thanks to the OCT-DVC method. Eventually, we proposed a model assuming that chemoelasticity affects only the hydrostatic pressure whereas the deviatoric stress only depends on the hyperelastic contribution of the tissue. Without doubt, chemoelasticity plays an essential role in arterial mechanobiology in vivo and future work should focus on characterizing chemoelastic effects in arterial walls under physiological and disease conditions.

Keywords: arteries, optical coherence tomography, digital volume correlation, full-field strain measurement, chemoelasticity, mechanobiology, aortic dissection.

Statement of significance: Chemoelasticity, coupling diffusion phenomena and mechanical stresses, is essential in soft tissue mechanobiology. For the first time ever, we measure and analyze 3D strain fields induced by these chemoelastic effects thanks to the unique combination of OCT imaging and digital volume correlation.
1. Introduction

Arteries, which carry our blood away from the heart to all organs, have three distinct tissue layers: the tunica intima, the tunica media, and the tunica adventitia. In large elastic arteries such as the aorta, the media is the thickest and most substantial layer. It comprises fascicles of irregularly shaped smooth muscle cells (SMCs) within a highly structured extracellular matrix (ECM), in which layers of elastin and collagen are embedded in a gel-like ground substance consisting of glycosaminoglycans (GaGs) and proteoglycans [1].

Whereas the biomechanical properties of large arteries have been an intense topic of research [2], transport of water across arterial walls is a topic which received significantly less attention [3][4]. An important transmural pressure gradient (100 mmHg) exists between the intraluminal arterial blood pressure and adventitial interstitial pressure, creating a unidirectional outward hydraulic conductance across the arterial wall. This hydraulic conductance is responsible for advective radial mass transport of soluble plasma molecules and macromolecules through the arterial wall, which depends: (i) on hemodynamic factors, including pressure and wall shear stress [5], (ii) on the porosity of the arterial wall, partly determined by the integrity of the elastic network, by its stretch and by the SMC tone which limit advection across the wall [1], (iii) on osmotic pressures in the wall and in the surrounding media. For example, free water molecules will be retained more in hydrophilic areas of the aortic wall than in hydrophobic ones (elastin), and could contribute to the swelling of the GAG-rich mucoid degenerative areas [6][7].

As the stretch of ECM affects outward advection, it is evident that the interstitial fluid also plays an important role on the deformability of soft biological tissues. Its ability to move through the interstitial space and across the boundary permits reversible changes of tissue volume through fluid exchange with the environment, which in turn is affected by the osmotic activity of the tissue constituents. These fluid motions are commonly neglected and this is often taken as an argument for tissue incompressibility. In the pioneering work of Carew et al. (1968), the arterial wall was assumed as a homogeneous substance consisting in semisolid fibrillar structures embedded in a gelatinous matrix, setting the basis for nearly incompressible hyperelastic models within the framework of continuum mechanics [8]. However, hydrated biological tissues
are intrinsically multiphasic, which has led to a number of challenging questions about the meaning of Poisson’s ratio and about its variations [9][10].

Hydration may also affect significantly the mechanical behavior of tissues. For instance, elastin needs to be highly hydrated to remain elastic [11]. Wang et al. (2018), recently showed that purified elastin treated with polyethylene glycol (hence with a significant dehydration, including loss of intrafibrillar water) became very rigid and was also significantly more viscoelastic [12]. Therefore, for a comprehensive approach of arterial mechanobiology, it is necessary to model fluid flow, variations in chemical potential and osmotic pressure in addition to stress and strain in the tissue. Such coupled phenomena of fluid flow and deformation which are typical of hydrated biological tissues are well treated within the theory of chemoelasticity [13][14], which was originally developed to address chemically driven swelling in geomechanics [15]. It has been well adopted for soft tissues such as cartilage, which contain a significant fraction of GaGs [16]. Lanir introduced a chemoelastic theory of arteries to explain residual stresses [17]. Following Lanir’s pioneering work, Azeloglu et al. (2008) investigated, both numerically and experimentally, the regulating role of proteoglycans present in the arterial wall, showing that, with an inhomogeneous distribution of proteoglycans through the wall thickness, the osmotic pressure would vary across the wall thickness, resulting in an inhomogeneous swelling stress field in the solid matrix, affecting significantly the opening angle observed experimentally [18].

Despite these advanced theoretical and computational studies, chemoelasticity in arteries still requires experimental insights. Among the manifold of experimental techniques, full-field optical measurement techniques, such as digital image correlation (DIC) or digital volume correlation (DVC) [19][20][21][22][23][24][25][26], have recently been extended to the mechanical behaviour of arteries and have revealed major regional inhomogeneities of strains and stiffness [27][28].

Additionally, and regarding the characterization of aortas, few studies have used the OCT technique to achieve full-field strain measurements [29]. Only Acosta Santamaria et al. (2017), applied a tensile test in conjunction with the OCT and DVC methods to obtain measurements of depth-resolved deformations across porcine aortic tissues immersed in a tissue clearing agent (propylene glycol - PG) [30][31]. The obtained OCT-DVC strain fields showed interesting inhomogeneities across the different layers of the aorta.
In the work reported here, similar experiments were repeated and analyzed thoroughly, showing that PG induces incidentally major chemoelastic effects on arteries which permit to observe fluid flow and their induced strains in arteries undergoing uniaxial tension [32]. The objective of the present paper is then to measure and interpret chemoelastic strain fields in arteries using OCT-DVC.

2. Materials and methods

2.1. Sample preparation

Four descending thoracic aortas were ordered from the Veterinary Campus of the University of Lyon (VetAgro Sup, Marcy l'Etoile). Youna castrated pigs, aged between 5 and 6 months and weighing between 80 and 90kg were sacrificed in accordance with the recommendations of the VetAgro Sup Ethics Committee (C2EA No. 18), and in accordance with the regulations on animal experimentation - Directive 2010/63 / EU. Aortas were excised and stored at −20°C. The postmortem autolysis was minimized by an immediate freezing of the tissue and by performing the tests soon after the specimens were thawed.

After thawing, and for all the experimental protocols implemented, the samples were immersed in an osmotically active solution one hour before and during the tests (see figure 1a). Propylene glycol (PG) and phosphate buffered saline (PBS) were mixed to prepare an osmotically active solution improving the optical properties of the tissue by dehydration. Accordingly, the obtained penetration depth and image contrast enabled measurements across the whole thickness of the aortic wall [30]. The PG, as an optical clearing agent, was previously used by our group. Details may be found in our previous publication [30].

All the aortic samples were obtained in a region between intercostal branches (see figure 1a). The length was aligned with the circumferential direction of the tissue (Y-Axis), and the width with the longitudinal direction (X-Axis), whereas the Z-Axis defined the thickness (see figure 1a).

In total, 11 aortic samples were prepared for different purposes:
- one sample (10 x 14 mm), named sample A1, was used to perform the OCT-DVC calibration using a rigid body motion test.
six samples (10 x 20 mm), named samples B1, B2, ..., B6, were used to evaluate the optical scattering properties of the aortic tissue immersed in different osmotically active solution concentrations.

- three rectangular samples (10 x 58 mm), named C1, C2 and C3, were immersed in an 80% PG solution and tested in uniaxial tension (stress-relaxation tests). Their thickness was measured at five different locations before and after the immersed condition (1.87 ± 0.28 and 1.44 ± 0.30 mm, respectively), and at the end of the experimental protocol (1.17 ± 0.4 mm). Strain fields were measured during these tests using the OCT-DVC technique described further.

- one rectangular sample (10 x 58 mm), named D1, was immersed in a 60% PG solution and tested in uniaxial tension for monitoring the evolution of the OCT signal without using the OCT-DVC method.

2.2. OCT acquisitions
A preliminary calibration of the OCT-DVC method was achieved on sample A1. A rigid body translation test was established to determine the optimal voxel size of OCT acquisitions. Linear translations were applied in the X-Axis (0, 20, 40 and 60 µm), and considering 5, 6, 7, 8 and 9 µm pixel sizes on the longitudinal-circumferential X-Y plane (OCT B-Scans). Other pixel sizes were explored in our previous publication (3, 4, 5 and 6 µm) [30]. For the Z-Axis the pixel size was 1.42 µm (OCT A-Scan). The field of view (FOV) was 2 x 3 x 1.48 mm (X, Y and Z-Axis, respectively).

Additionally, and to determine the incidence of the optical clearing agent on the optical properties of the tissue, several PG concentrations were considered on samples B1 to B6. The goal was to identify the minimum and necessary PG concentration to allow sufficient contrast for an entire through-thickness OCT acquisition. The concentrations varied between 30% and 80% v/v PG/PBS: 30/70 for sample B1, 40/60 for sample B2, 50/50 for sample B3, 60/40 for sample B4, 70/30 for sample B5 and 80/20 for sample B6. OCT acquisitions were performed with 6 µm pixel size on the X-Y plane and 2.45 µm pixel size in the Z-Axis. A FOV of 2 x 2 x 2.8 mm was defined.

Subsequently, the OCT signal intensity was acquired during uniaxial tensile tests [30] carried out on samples C1, C2 and C2 immersed in the 80% PG solution. A stepwise stress-relaxation ramp was applied and OCT volumetric images were acquired for each load-step during the maximum relaxation phases. The OCT parameters were: a voxel size of 5 x 5 x 2.45 µm and a FOV of 2 x 3 x 2.51 mm (X, Y and Z-Axis,
respectively). To ensure that the zone of interest deformed without significant rigid body motion, the FOV was increased of one millimeter in the Y-Axis (2 x 4 x 2.51 mm), see figure 1b. We used a Thorlabs OCT-TEL220C1 OCT system. The system features were: a center wavelength of 1300 nm, lateral resolution 7 µm, focal length 18 mm, maximum sensitivity range 111 dB (at 5.5 kHz). During the experiments, the OCT illumination tube was in full-contact with the osmotic solution and the lens was focused on the outer surface of the intima layer (corresponding to OCT B-scans), see figures 1a - 1b. The OCT acquisition datasets were saved in TIFF format. The TIFF virtual stack was imported using the ImageJ® software. The data were rescaled and converted to 8 bits for intensity levels digitalization. Finally, the data were exported as RAW images. With the RAW data, and applying the DVC method, displacement fields were measured. Finally, a similar uniaxial tensile tests was carried out on sample D1 immersed in the 60% PG solution. OCT volumetric acquisitions were acquired for each load-step but they were not processed with DVC as the signal intensity was not sufficient.

2.3. Uniaxial tensile test with stepwise stress-relaxation
Before carrying out the uniaxial tensile tests on samples C1, C2, C3 and D1, two preconditioning cycles were applied [33][34][35][36]. Both cycles considered a 14.8% deformation of the sample and return to the unloaded length. The initial distance between clamps was 35.1 mm (see figure 1a). The deformation was applied under displacement control and the displacement increment was 5.2 mm. The relaxation phase lasted 38 min, 30 min to define the equilibrium and 8 min to obtain the corresponding OCT acquisition. The criterion for a complete equilibrium was a relaxation rate <100 Pa/min [37].

After preconditioning, a stepwise stress-relaxation ramp was applied with nine controlled displacements and relaxation phases between increments of 38 min. The displacement ramp was defined in the circumferential direction of the tissue by increments of 0.6 mm (Y-Axis, corresponding to OCT B-scans), see figures 1b - 1c. In order to maintain the center of the sample in the same position, equal displacements were applied in two opposite directions simultaneously (0.3 mm on each side), see figure 1c. A final engineering strain of 28% was approximately reached. The load was monitored with a submersible load cell of 22 N (rated output +/-1.57 mV/V) conditioned with a Futek IPM650 panel mount display (input range up to +/-500 mV/V).
2.4. Strain measurements by Digital Volume Correlation (DVC)

3D displacement fields were measured using DVC with a local correlation algorithm (LA-DVC) [30][38][39][40]. Briefly, the reference configuration \( f \) can be represented by the gray-level function \( f(x, y, z) \), and the deformed state \( g \) as \( g(x + u, y + v, z + w) \), where \((x, y, z)\) represents the coordinates and \((u, v, w)\) the offset in each direction. Considering \((u, v, w)\) as the displacement mapping, the continuity of the gray-level can be assumed as \( f(x, y, z) = g(x + u, y + v, z + w) \) [40][41]. The DVC method was applied using the DaVis\textsuperscript{®} (LaVision) software. The sub-volume discretization and the multi-pass approach in DaVis\textsuperscript{®} were used to achieve the maximum cross-correlation coefficient (considering the gray-level distributions) [39][42][43][44].

The implemented correlation parameters were the same as in our previous work [30], but we added a uniform filter and a 2 x 2 x 2 binning volume. For the rigid body translation test on sample A1, the DVC method was performed at 3 different steps and permitted to reconstruct the displacement fields between the reference configuration and each of these translated configurations. In the uniaxial stress-relaxation test, for samples C1, C2 and C3, the DVC method was performed at 9 different steps and permitted to reconstruct the displacement fields between the reference configuration (defined after preconditioning) and each of these translated configurations.

Before performing DVC, four specific regions of interest were defined in the OCT volume images: the whole aortic wall (global), the intima region (layer I), the media region (layer M) and the adventitia region (layer A). After measuring the 3D displacement fields in each region, they were approximated by tricubic functions and the Green–Lagrange strain components were derived [45][46][47]. All the postprocessing approach was implemented in MatLab\textsuperscript{®}.

3. Results

3.1 OCT acquisitions

The OCT-DVC method was preliminarily optimized in terms of spatial resolution and contrast acquisition. For the spatial resolution, the 5 µm pixel size provided the smallest DVC errors, yielding displacements of 19.2 ± 3.12 µm, 39.9 ± 3.78 µm and 60.4 ± 4.64 µm, for applied translations of 20 µm, 40 µm and 60 µm, respectively. For the contrast, acquisitions across the entire aortic wall were available between 60% and 70% PG
concentration. Moreover, and for 80% PG, it is interesting to notice that the scattering is reduced significantly at the middle of the thickness (Figure 2). This region corresponds to the media layer of the aorta. In soft tissues, the scattering origin is attributed to the long collagen and elastin fibers and the extracellular medium which have a different refractive index than interstitial and intracellular water. After dehydration of the media the refractive index becomes nearly uniform.

The uniaxial tensile test on sample D1, immersed in a 60% PG solution permitted to assess how the OCT signal varies with the applied deformation. Figure 3 shows the OCT acquisitions for four of the ten load-steps on sample D1. The initially dark region at the middle of the thickness gradually faded away as the tissue recovered its scattering properties when the stretch increased (see figure 3). This was induced by tissue rehydration and swelling. Due to the applied tension, the osmotic pressure in the tissue was increased (reverse osmosis) and a new fluid flow equilibrium across the tissue was defined. In this context, a concentration of 60% PG does not achieve the optimal contrast and depth capability to measure 3D displacement fields across the whole aortic wall. Consequently, displacement fields could only be measured for samples C1, C2 and C3 which were immersed in an 80/20 (v/v - PG/PBS) solution.

3.2 Response curves
When carrying out the tensile test, temporal evolutions of the force showed a significant relaxation corresponding to the equilibrium of the osmotic effects (see figure 4a). For samples C1, C2 and C3, immersed in 80% PG, the reported force values were at least 5 times larger than the ones for sample D1, immersed in 60% PG. The osmotic effects are known to induce an additive stiffness, called the osmotic stiffness. These effects were significantly larger for the 80% PG concentration. Moreover, stress-strain curves were plotted, using the force values reached at the end of relaxation. The behavior highlights the significant increase of stiffness induced by the osmotic effects with 80% PG concentration (see figure 4b). The tangent elastic modulus was estimated in a range of strains between 0.15 and 0.2. It was about 1.35 MPa in 80% PG and 0.07 MPa in 60% PG. Therefore, the elastic modulus with the 60% PG concentration is ≈5% of the one reported with 80% PG. Values obtained in 60% PG are in good agreement with mechanical properties reported by Choudhury et al. (human samples immersed in a saline bath) and Wang et al. (mouse aged samples) [48][49].
3.3 Strain fields
Thanks to the OCT-DVC method, the strain fields could be measured in each sample throughout the different loading stages. The reference configuration was defined as the configuration reached after preconditioning.
In figure 5, we show the distributions of $\varepsilon_{xx}$, $\varepsilon_{yy}$ and $\varepsilon_{zz}$ across the transverse plane normal to the loading axis located at the middle of the region of interest. As expected in a tensile test, $\varepsilon_{xx}$ and $\varepsilon_{yy}$ had rather uniform distribution, with $\varepsilon_{yy}$ being positive (tensile strain) and $\varepsilon_{yy}$ being negative (transverse strain). However, $\varepsilon_{zz}$ always showed a very specific pattern, with negative strains at the top and bottom of the region of interest (intima and media layers, respectively), and positive strains at the middle of the thickness (media layer). The positive $\varepsilon_{zz}$ is counterintuitive for a tensile test as a usual Poisson’s effects in a tensile test would induce negative transverse strains along the Z-Axis. However, the positive $\varepsilon_{zz}$ is in agreement with the osmotic effect observed earlier, with fluid flowing back into the media layer when the strain increases, thus inducing a swelling of the tissue at the middle of the thickness.

3.4 Evolution of average strains
Interestingly, when we plot the average strain across the whole zone of interest (see figure 6), $\varepsilon_{zz}$ does not vary and remains almost zero when the stress increases, $\varepsilon_{yy}$ reaches values between 0.12 and 0.14, and $\varepsilon_{xx}$ reaches values between -0.02 and -0.04. Assuming that $\varepsilon_{xx}+\varepsilon_{yy}+\varepsilon_{zz}$ defines the change of volume locally, there is a global increase of volume of nearly 10%, which may be explained by the swelling effect. Although $\varepsilon_{yy}$ and $\varepsilon_{xx}$ had nearly the same values in each of the three layers (see figures 7a – 7b), $\varepsilon_{zz}$ showed a very specific response with negative strains reaching between -0.06 and -0.08 in the intima and in the adventitia, and positive strains reaching about 0.02 in the media layer (see figure 7c). This means that the volume increase was mainly concentrated in the media, reaching 12%, whereas it was less than 5% in the intima and in the adventitia. This shows that most of the osmotic effects occurred within the media with a significant uptake of fluid during the tensile test.

4. Discussion
In this study, we carried out uniaxial tensile tests on arteries immersed in a hyperosmotic solution exacerbating chemoelastic effects, and we were able to measure different manifestations of these chemoelastic effects: existence of an osmotic modulus on top of the elastic modulus, swelling of the media inducing a
modification of its optical properties, existence of a transverse tensile strain (negative Poisson’s ratio). For the first time ever to our best knowledge, 3D strains induced by these chemoelastic effects in soft tissues were quantified thanks to the OCT-DVC method. It should be noticed first that immersion in a hyperosmotic solution (80% PG) led to a significant dehydration of the tissue, permitting to image its reflectance across the whole arterial thickness with the OCT method and thus enabling the 3D strain measurements using DVC. Dehydration is a commonplace technique for imaging soft tissues. The highly scattering nature of nontransparent human tissue limits the imaging depth of optical coherence tomography (OCT) to 1–2 mm. When longer wavelengths are used, the penetration depth can be improved; however, the imaging contrast is decreased, largely because of reduced backscattering at the microscopic scale and reduced refractive heterogeneity of the macroscopic scale. To enhance the penetration depth and imaging contrast in OCT imaging, the use of PG is rather efficient [32]. A hyperosmotic agent such as PG can draw fluid out of the tissue by creating an osmotic pressure difference between the immersion solution and the arterial fluids. The osmotic pressure inside the arterial tissue is partly regulated by GAGs and proteoglycans, which control hydration and water homeostasis in arteries and which majorly reside in the media [50]. The osmotic pressure of inflated GAGs induces tensile loading of the fibrous network (elastin, collagen), which in turn entangles/restrains the GAGs within the ECM, as well as provide shear stiffness to the ECM and help maintain ECM organization [51]. Out of a hyperosmotic agent, GAGs permit to maintain an osmotic pressure larger inside the tissue than outside, keeping water inside the tissue. Although the global tensile response is changed in presence of chemoelastic effects, it may be assumed that the intrinsic elastic properties of the tissue are not altered. To account for the chemoelastic effects, the arterial tissue should first be modelled as a biphasic medium and equilibrium conditions should be considered conventionally in an NaCl bath. The Cauchy stress of the biphasic medium, made of a solid component and an interstitial fluid component, is assumed to be written such as:

$$\mathbf{T} = -p \mathbf{I} + \mathbf{T}^e$$

(1)

Where:

$-p$ is the osmotic pressure difference between the interstitial fluid and an external salt solution (NaCl bath) and $\mathbf{T}^e$ is the Cauchy stress of the solid component, which is assumed to be incompressible hyperelastic, and then for which the Cauchy stress may
be written as the sum of contributions of a NeoHookean matrix and contributions of a number of fiber families having an exponential stress strain relationship [10][52]:

\[ \mathbf{T}^e = J^{-1} \left[ k_0 \ \text{dev} (\mathbf{F} \mathbf{F}^T) + \sum_n k_{1n} \exp \left( k_{2n} \left( (\mathbf{F}^T \mathbf{F}) : \mathbf{A}_n - 1 \right)^2 \right) \ \text{dev} (\mathbf{F} \mathbf{A}_n \mathbf{F}^T) \right] \quad (2) \]

Where:
\( \mathbf{F} \) is the deformation gradient tensor, \( J \) is the change of volume (determinant of \( \mathbf{F} \)), \( \mathbf{A}_n \) are the structural tensors of fiber directions and \( k_0, k_{1n}, k_{2n} \) are material constants.

At equilibrium, it can be shown for a dilute solution that the osmotic pressure difference between the interstitial fluid and a NaCl bath may be written [17]:

\[ p - p^* = R \theta \left[ c^F + (c^F)^2 / (2\bar{c}^*) \right] \quad (3) \]

With:

\[ c^F = \varphi_0^w c_0^F / (J - 1 + \varphi_0^w) \quad (4) \]

Where:
\( p^* \) is the ambient pressure in the NaCl bath (taken to be zero), \( R \) is the universal gas constant, \( \theta \) is absolute temperature, \( c_0^F \) and \( \varphi_0^w \) are the fixed-charge (GaGs) density and water content in the strain-free reference configuration, \( \bar{c}^* \) is the external NaCl bath salt osmolarity (\( \bar{c}^* = 2c^* \), where \( c^* \) is the salt concentration in the NaCl bath).

As in our case, the artery is equilibrated against a solution of PG which cannot permeate into the tissue, then [53]:

\[ \mathbf{T} = -(p - \pi) \mathbf{I} + \mathbf{T}^e \quad (5) \]

Where \( \pi \) is the osmotic pressure difference between the PG solution and the NaCl bath. Following Eq. 3, it can be assumed that:

\[ \pi = R \theta \left[ c^{PG} + (c^{PG})^2 / (2\bar{c}^*) \right] \quad (6) \]

Finally, the Cauchy stress is rewritten:

\[ \mathbf{T} = R \theta \left[ c^{PG} - \frac{\varphi_0^w c_0^F}{J - 1 + \varphi_0^w} + \frac{1}{2\bar{c}^*} \left( (c^{PG})^2 - \left( \frac{\varphi_0^w c_0^F}{J - 1 + \varphi_0^w} \right)^2 \right) \right] \mathbf{I} \]

\[ + J^{-1} \left[ k_0 \ \text{dev} (\mathbf{F} \mathbf{F}^T) + \sum_n k_{1n} \exp \left( k_{1n} (\mathbf{C} : \mathbf{A}_n - 1)^2 \right) \ \text{dev} (\mathbf{F} \mathbf{A}_n \mathbf{F}^T) \right] \quad (7) \]

The first term represents the osmotic effects on the stress and the second the hyperelastic effects. The first term shows that:
1. the higher the concentration of PG, the larger the contribution of osmotic effects on the Cauchy stress
2. the partial derivative $\partial T/\partial J$ is positive (osmotic modulus), showing that swelling $(J > 1)$ induces a significant stress increase.
3. for the largest concentration of PG (80%), the tissue is nearly dehydrated and we can assume $\phi_0^w \approx 0$. The osmotic modulus factorizes by $1/(J - 1)^2$ which explains the very large osmotic stiffness in such situation, as seen in figure 4.

Whereas the osmotic effects affect only the hydrostatic pressure in the total Cauchy stress, we assume that the deviatoric stress remains fully independent of osmotic effects and only depend on the hyperelastic contribution of the tissue. Therefore, if one can isolate the deviatoric contribution from the hydrostatic pressure, for instance by using the virtual fields method [28][54], it is still possible to characterize the hyperelastic material properties of the tissue despite the osmotic effects induced by the PG immersion. It is worth mentioning that the effect of dehydration was also examined by other authors in porcine aortic valve cusps [55]. Shear testing was performed on physiologically hydrated, superhydrated, and dehydrated cusps. The effect of altered hydration on shear properties was significant and followed trends which are consistent with the ones reported in our study.

A higher PG concentration improved the optical scattering properties and depth capability (see figure 2). PG dehydrated the tissue extracting the interstitial and intracellular water content. The thickness decreased by 23.10% and 37.64% after the immersion and at the end of the uniaxial tensile test, respectively. For Wang et al. (2018), the thickness of the aortic tissue was more sensitive to water loss and decreased to almost half of the original value using a higher solute concentration (polyethylene glycol - PEG) [12].

In this work, we only measured the steady-state strains, when the tissue had reached a chemoelastic equilibrium. The steady state situation was reached after a transient period during which fluid motions induced a relaxation response of the tissue, similar to viscoelasticity. We noticed that the duration of relaxation was increased with higher PG concentration, see figure 4. Shahmirzadi et al. (2013) also showed that relaxation effects in a dehydrated tissue (immersed in Polyethylene Glycol) were slower than in a hydrated tissue (native - bovine thoracic aortas) [56]. Wang et al. (2018 a - b) showed that relaxation was increased after removing extrafibrillar water from isolated elastin
extracted from porcine thoracic aorta [12]. Similarly in isolated elastin, a glucose treatment was also shown to increase relaxation [57].

However, no one before us had measured the steady-state 3D strain fields related to osmotic effects in soft tissues. The full-field measurements performed on the aortic wall immersed in PG highlight the complexity of chemoelastically induced strains. The biomechanical behavior of arterial walls is known to be complex and anisotropic due to its fibrous nature (elastin and collagen) and due to its layered structure (intima, media & adventitia). Chemoelastic effects bring another layer to this complexity. This results in very peculiar Poisson’s effects. An original aspect of the local mechanical behavior revealed by OCT-DVC method was the heterogeneity of Poisson’s effects across the thickness. The intima and the adventitia showed the major Poisson’s effect on the mechanical behavior with corresponding strains of 4.58% and 3.80%, respectively (which are values expected for a nearly incompressible material).

Conversely, the media layer showed a tensile transverse strain (effect of swelling), equivalent to a negative Poisson’s ratio. Such effects induced by chemoelasticity were already reported for thin membranes [10].

These results permit a better understanding of soft tissue biomechanics. However, they were obtained in vitro with very specific testing conditions. There remains a very important gap towards understanding and characterizing osmotic effects in vivo. It is known that water filtration across the aortic wall is essential for the normal physiological function of the circulation and its alteration may have important mechanobiological consequences [5]. Moreover, swelling of the arterial wall may even become detrimental as alterations in the hydro-seal might also play a role in the dissection of arterial walls when the distribution of pressures across the wall is not properly controlled [7].

Although the water permeability of the intact arterial wall has been extensively characterized [58][59][60][61][62], the precise location and biochemical composition of the water permeation barrier, which are fundamental properties of the arterial wall, are still partially unknown and should be investigated further to better account for the essential role of chemoelasticity in arterial mechanobiology.

6. Conflict of interest
The authors declare they have no conflict of interest related to this work.

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Figure 1.
Figure 2.

A-Scan 3D - OCT
(30/70) PG-PBS
(40/60) PG-PBS
(50/50) PG-PBS
(60/40) PG-PBS
(70/30) PG-PBS
(80/20) PG-PBS

PG Concentrations (%)

FOV: 2 x 2 x 2.8 mm
Pixel Size: 6 x 6 x 2.45 µm
Figure 3.
Figure 4.

Load-relaxation Uniaxial Tensile Test

Experimental Force (N) vs. Time (min)

Load-relaxation... 0.19 0.21 0.23 0.25 0.27 0.29

σ (MPa) vs. Strain (Ɛ)

Linear Elastic Range

Pre-condition Relaxation Time

Stress - Strain

σ (MPa) vs. Ɛ

Linear Elastic Range

Figure 4.
Figure 5.

Global, entire aortic wall; I, intima; M, media; A, adventitia.
Figure 6.

Normal Strain Components ± SD
Entire Aortic Wall - Global Behavior

Experimental Force (N)

$\varepsilon$

1 $\varepsilon_{xx}$  1 $\varepsilon_{yy}$  1 $\varepsilon_{zz}$
2 $\varepsilon_{xx}$  2 $\varepsilon_{yy}$  2 $\varepsilon_{zz}$
3 $\varepsilon_{xx}$  3 $\varepsilon_{yy}$  3 $\varepsilon_{zz}$
Figure 7.