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Pressure-induced collagen degradation in arterial tissue as a potential mechanism for degenerative arterial disease progression

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ABSTRACT

Collagen fibre degradation is a strain-dependent process, whereby the magnitude of experienced strain dictates the rate of enzymatic cleavage. Studies have identified conflicting findings as to whether strain inhibits or enhances collagen degradation, which may be explained by the tissue type and tissue scale investigated, as well as the strain range considered. The aim of this study is to identify, for the first time, the strain-dependent degradation response of intact arterial vessels experiencing physiological pressures and apply these findings to a computational model to better understand degenerative arterial diseases, such as aneurysms.

To achieve this, a series of quasi-static pressure inflation experiments were carried out on intact arteries in the presence of purified bacterial collagenase at physiologically relevant pressures to investigate collagen matrix degradation in the vascular wall. A complementary computational model was developed to explore the complex role of pressure, non-collagenous matrix contribution, and collagen fibre crimp in the ultimate degradation response of the vessel.

Pressure induced inflation-degradation results identified an increased rate of vessel expansion and reduced time to failure with increasing pressure in the vessels. Interestingly, our computational model was able to capture this same response, including the elevated rates of degradation which occur at low pressures. These findings highlight the critical role of strain in collagen degradation, particularly in cases of arterial disease, such as aneurysm formation, whereby structural integrity may be compromised.

1. Introduction

Collagen fibre remodelling, consisting of reorientation, production and degradation of collagen is central to healthy and diseased arterial remodelling. Collagen degradation, in particular, is critical in dictating the mechanical stability of collagenous tissues, with excessive degradation linked to atherosclerotic plaque cap rupture (Aikawa, 2004; Daemen et al., 2016) as well as aneurysm progression and rupture (Thompson et al., 2002). Collagen fibre degradation has also been identified as a strain-dependent process where strain has been found to enhance (Ellsmere et al., 1999; Willett et al., 2008) as well as inhibit (Lotz et al., 2008; Nabeshima et al., 1996; Robitaille et al., 2011; Ruberti and Hallab, 2005; Wyatt et al., 2009; Zareian et al., 2010) collagen

degradation. A greater understanding of this strain-driven remodelling response may provide fundamental insights into arterial disease progression and the biomechanical responses following medical device deployment.

Our previous work (Gaul et al., 2018) uncovered an underlying V-shaped degradation response in arterial collagen, where strain initially reduces the degradation rate before once again increasing above a strain protective threshold. This response was partially masked at the tissue scale by the mechanical contribution of non-collagenous matrix and a transmural gradient of collagen fibre crimp. A complementary 1D numerical model supported this finding, highlighting the importance of this non-collagenous matrix and crimp gradient in the ultimate degradation response observed at the tissue scale. However, these findings

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were determined in excised dogbone specimens, which have an altered gradient of collagen fibre crimp and altered residual stresses compared to physiologically relevant native vessel geometries (Gaul et al., 2018; Holzapfel et al., 2007). These structural changes may affect the degradation response observed experimentally *in vitro*. Understanding the physiologically relevant tissue scale response of intact vessels is therefore critical in understanding and treating diseases which, if left unchecked, may lead to an adverse clinical event (Thompson et al., 2002).

A number of studies have identified a similar V-shaped collagen degradation profile in response to applied strain (Ghazanfari et al., 2016a; Huang and Yannas, 1977; Yi et al., 2016). Interestingly, the strain at which a minimum degradation response occurs was found to be significantly different depending on the tissue scale investigated. Studies on reconstituted collagen tapes identified a collagen protection mechanism at approximately 3.5% strain (Huang and Yannas, 1977) in contrast to approximately 20% strain in bovine pericardium (Ghazanfari et al., 2016a), lung tissue (Yi et al., 2016) and artery (Gaul et al., 2018). It has been suggested that this degradation response may be influenced by moving from the nanoscale to the macroscale due to molecular, fibril and fibre sliding, unfolding, reorientation and straightening (Chang et al., 2012; Chang and Buehler, 2014; Gaul et al., 2018; Willett et al., 2008). Similarly, packing of collagen molecules into fibrils and fibres may influence the degradation response further by increasing the structural stability of the collagen (Miles and Ghelashvili, 1999; Willett et al., 2008).

A number of computational models have been developed in order to more thoroughly investigate the collagen degradation response observed experimentally (Gaul et al., 2018; Heck et al., 2015; Loerakker et al., 2014; Tonge et al., 2015) as well as predict disease development and progression (Grytz et al., 2012; Loerakker et al., 2016). Using an energy-based approach, Tonge et al. were able to capture the degradation response of bovine pericardium (Ellsmere et al., 1999) and corneal tissue (Zareian et al., 2010) observed experimentally through a reduction in collagen fibre radius (Tonge et al., 2015). Using a strain-based approach resulting in a collagen volume change, Loerakker et al. were also able to predict the remodelling response of tissue engineered heart valves *in vivo*, showing increased likelihood of valvular insufficiency when valves are implanted in the low pressure pulmonary position in comparison to the aortic valve position (Loerakker et al., 2016). Degradation models have also been used to predict thickening of lamina cribrosa, a connective tissue structure supporting axons exiting the eye, which occurs in early glaucoma and has been associated with maladaptive remodelling (Grytz et al., 2012). Similar degradation models may help elucidate maladaptive remodelling of collagen in arterial diseases, which may ultimately be responsible for plaque and aneurysm weakening and rupture (Aikawa, 2004; Bersi et al., 2019; Daemen et al., 2016; Thompson et al., 2002). However, before inferring conclusions from computational simulations of arterial diseases, it is important to confirm that our previous findings describing collagen degradation for excised arterial dogbone specimens (Gaul et al., 2018) are also applicable in the case of intact vessels, under physiological pressures and with residual stresses.

Therefore, the aim of this study is to determine the strain-dependent degradation response of collagen in intact arterial vessels at physiologically relevant blood pressures and to show a case study exhibiting how these experimental findings can be adopted to inform computational models for arterial disease. To achieve this, the study had the following specific aims; 1) to propose and employ an experimental method to investigate the creep response of intact vessels as they degrade in the presence of collagenase at constant pressures, and 2) to develop a 3D finite element model based on our findings and our previous study (Gaul et al., 2018), to explain our experimental creep data.

2. Methods

2.1. Tissue preparation

Fresh porcine common carotid arteries were excised from 6-month-old Large White pigs ($n = 30$) weighing approximately 80 kg at the time of slaughter. Excised carotid arteries were taken from the proximal end of the vessel (Trabelsi et al., 2019) and then frozen to -80°C at a controlled rate of $-1^{\circ}\text{C}/\text{min}$ in a cryoprotectant media to maintain mechanical and structural properties during storage (Müller-Schweinitzer, 2009). Prior to testing, vessels were thawed in a water bath at 37°C to minimise tissue damage (Pegg et al., 1997), washed in PBS to remove any residual cryoprotectant and cut to approximately 30 mm in length for testing. Loose connective tissue was carefully removed using a forceps and scalpel. This served a dual purpose: it facilitated the easy application of a speckle pattern for accurate surface strain measurement, and produced a more uniform specimen geometry for structural analysis. Each specimen was then cannulated using nylon barbs and tied using nylon thread. A speckle pattern, which is required for digital image correlation (DIC) strain analysis, was then applied using black alkyd resin spray paint from a distance of approximately 70 cm. Unlike ink dots, that are often applied directly to tissue for strain tracking, which can dehydrate the tissue, spray paint ink effectively dries as it lands on the tissue, minimising potential tissue dehydration which may influence mechanical results. In order to mitigate the risk of pattern disruption during testing, the following procedure was performed: 1) sample was blotted dry, 2) speckle pattern was applied, 3) loose paint flakes were blotted off with tissue, 4) sample was placed in PBS to wash off any other loose paint flakes, 5) sample was blotted one final time to remove any remaining loose flakes before testing.

2.2. Pressure inflation experiments

2.2.1. Pressure inflation setup

To assess the structural response of intact vessels to collagen degradation, pressure inflation tests were carried out in the presence of purified bacterial collagenase. To achieve this, one end of the cannulated vessel was connected to a Proportional Integral Derivative (PID) controlled syringe pump and the other end was sealed with a cap, allowing for free axial expansion under inflation (Fig. 1). A pressure transducer was then inserted through a valve fitting enabling a constant pressure to be maintained as the vessel degrades. Once connected, the vessel was placed in a PID temperature-controlled water bath at 37°C . The pressure feedback system was controlled via LabVIEW (National Instruments, Berkshire, UK). To reduce the system complexity and allow meaningful conclusions on the strain-dependent collagen degradation mechanism in arteries to be determined, no axial strain was applied to the vessel, although axial tethering is known to be present *in vivo*. This condition was applied to ensure that circumferential strain remains the principal strain direction, even at low pressures, and to ensure that collagen fibres remain predominantly orientated in the circumferential direction throughout the experiments.

2.2.2. Strain measurement

Strain was tracked throughout degradation using a commercially available stereo camera Digital Image Correlation (DIC) system (Dantec Dynamics, Ulm, Germany), allowing for 3D strain mapping (Fig. 2). Prior to testing, the system was calibrated in a water bath to mimic testing conditions. Calibration was performed using a checkerboard plate of known dimensions supplied by Dantec Dynamics. The plate was tilted in all planes with 8–10 images being acquired through the DIC cameras. This allows the software to determine the angle between the cameras and the distance to the sample. The checkerboard calibration was carried out in solution at the location of sample testing to ensure the diffraction of light caused by the solution was accounted for.

After specimen testing, circumferential strain data was determined

Fig. 1. Schematic showing experimental setup consisting of a PID controlled pressure pump to maintain constant pressure during vessel degradation. Vessel degradation is measured through DIC analysis of a speckle pattern on the artery surface throughout degradation in a PID temperature-controlled water bath containing purified bacterial collagenase.

Fig. 2. Representative images of DIC analysis carried out on arteries in purified bacterial collagenase A) unpressurised at 0 min, B) pressurised to 180 mmHg at 10 min and C) pressurised to 180 mmHg at 60 min. Top panel shows raw images, bottom panel shows circumferential strain overlaid.

across the central portion of the vessel as shown in Fig. 2. Vessel edges were not included in the analysis due to the increased likelihood of edge effects influencing measurements, and the increased likelihood of these areas being out of focus as the furthest regions from the cameras.

The resulting strains were exported and post-processed in Matlab (MathWorks, Cambridge, UK) to determine the mean change in diameter in the circumferential direction as the vessel degrades at a given pressure. Strain measurements acquired using the DIC system were compared to a subset of diameter measurements, determined using ImageJ, for system validation (Schneider et al., 2012). Although DIC provides a full non-homogenous strain field across the vessel wall, the mean circumferential strain across the vessel surface was calculated in order to compare the degradation response at different pressures. Wall thickness of all vessels was determined by taking a ring section from the ends of each vessel and using a measuring microscope with a micrometer stage.

2.2.3. Pressure expansion tests

Once set up, purified bacterial collagenase (400 U/ml; Worthington Biochemical, USA) in PBS supplemented with calcium and magnesium (D8662; Sigma-Aldrich, Ireland) was added to both the syringe and the water bath. Each specimen was then pressurised to a fixed, constant pressure until either vessel rupture or the entire collagenase filled syringe volume (16 ml) permeated through the vessel wall and the syringe emptied. Each vessel was subjected to just one pressure. Different vessels were pressurised from 60 mmHg to 180 mmHg in 40 mmHg increments to determine vessel degradation across a large physiological pressure range. The experimental setup is depicted in Fig. 1. Three specimens were tested at each fixed pressure in the presence of collagenase, while one control vessel was evaluated at each pressure in the absence of

collagenase. Additionally, separate pressure-diameter curves were determined across 5 vessels for model calibration. The current setup did not allow the separation of expansion due to degradation and that due to the viscoelasticity of the arterial tissue. This was due to time constraints which did not permit the application of the speckle pattern after pre-conditioning of the vessel to remove viscous effects, i.e. it would take too long to apply the speckle pattern that the viscous effects would no longer be removed and the sample would have to be preconditioned again. By evaluating the timescale at which viscous creep was observed to occur in control vessels (approximately 90% of final recorded value at 10 min), however, it was clear that it was not necessary to separate these effects when evaluating pressure-induced degradation trends in arterial tissue, as degradation occurs at a considerably longer timescale than the viscous effects.

2.2.4. Investigation of low pressure degradation

To comprehensively investigate the degradation response at low pressures, that is, where minimal diameter changes occur, an additional set of pressure inflation experiments were carried out in a similar fashion to those outlined in Section 2.2.3. The objective of these experiments was to determine whether there is a higher rate of collagen degradation at low pressures, thus investigating the low strain region of the V-shaped degradation profile. Vessels were first pressurised in purified bacterial collagenase to either 60 mmHg or 100 mmHg for 45 min to allow for collagen degradation to occur. Vessel pressure was then removed before a pressure of 180 mmHg was reapplied while minimising any downtime. The time to failure was recorded and was used as a measure of how much collagen degradation had occurred at low pressure. The shorter the time to failure, the more the collagen that had been degraded at a specific pressure in the initial 45-min period.

2.3. Histological processing

Degraded vessel sections and undegraded adjacent sections of vessels were processed for histology to confirm collagen degradation. All samples were fixed in 10% formalin overnight at 4 °C, dehydrated in a graded series of ethanol baths (EtOH), cleared in xylene before being embedded in paraffin wax. The resulting paraffin-embedded samples were sectioned into 8 µm slices using a microtome (RM-2125RT, LEICA, Germany) and affixed to microscope slides. Slides were stained with picrosirius red and viewed under polarised light to evaluate collagen degradation. Picrosirius red enhances the natural birefringence of collagen allowing one to only see collagen when viewed under polarised light (Fig. 3) (Junqueira et al., 1979). The absence of collagen is clearly visible under polarised light by the absence of birefringence (appears as a completely black image when all collagen is absent).

2.4. Model development

To model vessel degradation under constant pressure, we modified a 1D constitutive law used to model collagen degradation in layered soft tissues (Gaul et al., 2018) for use in 3D finite element simulations. The constitutive law comprises an anisotropic collagen fibre component and an isotropic non-collagenous matrix component accounting for constituents such as elastin, glycosaminoglycans and the passive response of smooth muscle cells. Although elastin fibre directionality has been observed, it is far less aligned than that of collagen while also being less mechanically significant (Krasny et al., 2017). A more thorough description of the model can be found elsewhere (Gaul et al., 2018). The total Cauchy stress, σ_t , at a material point is given by:

$$\sigma_t = \sigma_f + \sigma_m \quad (1)$$

where σ_f and σ_m are the fibre and matrix stress tensors respectively.

Collagen fibre crimp, which can be seen experimentally in unpressurised arteries (see Fig. 3), is modelled by using a multiplicative split of the total stretch, λ_t , into the stretch of a collagen fibre, λ_f , and a precrimp component, λ_p .

$$\lambda_t = \lambda_f \lambda_p. \quad (2)$$

Precrimp, λ_p , quantifies the degree of crimp or pretension experienced by a collagen fibre and is a prescribed constant at the beginning of the simulation and does not change as load is applied. To set collagen fibres to be crimped in the initial configuration one must prescribe a $\lambda_p > 1$, such that a $\lambda_f < 1$ emerges. Likewise, if $\lambda_p < 1$ is prescribed, collagen fibres begin in tension in the initial configuration. Importantly, collagen fibres are assumed to only bear load in tension, and thus, do not contribute to the stress until a $\lambda_f > 1$.

Unlike our previous degradation model (Gaul et al., 2018), and more similar to (Loerakker et al., 2014), collagen fibres have an exponential stress-stretch relationship as proposed by Driessen et al. (2007) which is scaled by the collagen volume fraction:

$$\sigma_f = \begin{cases} \varphi_f k_1 (\lambda_f)^2 \left(e^{k_2 ((\lambda_f)^2 - 1)} - 1 \right) [a \otimes a] & \lambda_f \geq 1 \\ 0 & \lambda_f < 1 \end{cases} \quad (3)$$

where φ_f is the collagen volume fraction, k_1 and k_2 are collagen fibre stiffness parameters calibrated to experimental data, λ_f is the collagen fibre stretch for a given collagen crimp, λ_p , and tissue stretch, λ_t , defined in Equation (2) and a is a vector representing the fibre direction in the deformed configuration and the operator $a \otimes a = aa^T$. In this study we use a single family of circumferential collagen fibres, as observed in the porcine carotid artery (Gaul et al., 2017; Sáez et al., 2016; Shahid et al., 2017), unlike other vessels (Schriebl et al., 2012).

The non-collagenous matrix contribution to the total stress was modelled using a Neo-Hookean material [Loerakker et al., 2014] and scaled by its volume fraction:

$$\sigma_m = \varphi_m \left(\kappa \frac{\ln(J)}{J} \mathbf{I} + \frac{G}{J} (\mathbf{B} - J^{2/3} \mathbf{I}) \right), \quad (4)$$

where φ_m is the non-collagenous matrix volume fraction, $J = \det(\mathbf{F})$, $\mathbf{B} = \mathbf{F} \cdot \mathbf{F}^T$, $\mathbf{F}$ is the deformation gradient tensor, and κ and G are the bulk and shear modulus, respectively. The contribution of fibre and matrix stress which are weighted by their volume fractions, φ_f and φ_m , respectively, sum to unity.

2.4.1. Stress degradation

Collagen degradation was modelled by reducing the collagen volume fraction over time based on the collagen fibre stretch using a first order rate equation,

$$\frac{d\varphi_f}{dt} = -\Psi_f \varphi_f, \quad (5)$$

where Ψ_f is the degradation rate constant function.

As the collagen volume fraction reduces, so too does the collagen stress contribution as outlined in Equation (3). Collagen degradation was described by a V-shaped degradation profile based on our own findings (Gaul et al., 2018) and findings in literature (Ghazanfari et al., 2016a; Huang and Yannas, 1977; Yi et al., 2016), where stretch initially reduces the degradation rate before degradation once again increases above a protective stretch threshold (Fig. 4). This V-shaped profile was captured using a smoothed bilinear function with three domains; low strain, transition strain, and high strain (O'Connor et al., 2017). The low and high linear domains are connected using a quadratic function in the transition domain. Consequently, the collagen fibre degradation rate, Ψ_f , is described by the continuous stepwise function,

$$\Psi_f = D_c + \begin{cases} D_l \varepsilon_f, & \varepsilon_f < \varepsilon_{n1}^D \\ T_1^D \varepsilon_f^2 + T_2^D \varepsilon_f + T_3^D, & \varepsilon_{n1}^D \leq \varepsilon_f \leq \varepsilon_{n2}^D \\ D_h (\varepsilon_f - \varepsilon_{n2}^D) + T_1^D \varepsilon_{n2}^{D2} + T_2^D \varepsilon_{n2}^D + T_3^D, & \varepsilon_f > \varepsilon_{n2}^D \end{cases} \quad (6)$$

Fig. 3. Histological image of picrosirius red stained artery cross section viewed under A) light at 2x and B) polarised light at 40x showing a gradient of collagen fibre crimp through the thickness of an unpressurised vessel with greatest crimp observed at inner luminal surface. Undulation is also seen to increase in the adventitial tissue layer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Underlying V-shaped collagen degradation response, Ψ_f , for a single collagen fibre (Gaul et al., 2018). Degradation constants for given curve can be found in Table 1.

where D_l and D_h are the degradation moduli of the low strain and high strain regions respectively, the Green strain of the fibre, $\varepsilon_f = (\lambda_f^2 - 1)/2$; ε_{t1}^D is the strain at which the low strain region switches to the transition region; ε_{t2}^D is the strain at which the transition region switches to the high strain region; and D_c is a constant or baseline degradation rate which translates the bilinear curve along the y-axis. The parameters T_1^D , T_2^D , and T_3^D ensure continuity between the three regions and are defined as,

$$\begin{aligned} T_1^D &= (D_l - D_h) / (2(\varepsilon_{t1}^D - \varepsilon_{t2}^D)) \\ T_2^D &= D_l - \varepsilon_{t1}^D (D_l - D_h) / (\varepsilon_{t1}^D - \varepsilon_{t2}^D) \\ T_3^D &= D_l \varepsilon_{t1}^D - T_1^D \varepsilon_{t1}^{D^2} - T_2^D \varepsilon_{t1}^D \end{aligned} \quad (7)$$

To ensure conservation of mass, degraded collagen fibres are converted into a mechanically weak component using a reduction factor, R . As a result, Equation (4) is updated to account for this degraded material:

$$\sigma_m = \varphi_m \sigma_m + R \sigma_f (1 - \varphi_f - \varphi_m) \quad (8)$$

The second term on the right-hand side of Equation (8) accounts for the degraded material which no longer contributes to the stress in the tissue, with the expression in brackets (volume of degraded material) ensuring that the total volume fraction equals unity.

2.4.2. Precrimp gradient

The value of λ_p is varied across the artery wall as a function of the

radius r . To identify the idealised values of λ_p , we hypothesise that the gradient of collagen fibre crimp is minimised and the vessel strain is in a strain protection region at physiological pressure (100 mmHg/13 kPa). In order to determine the average crimp gradient for an artery in the unpressurised state based on this hypothesis, the following process was carried out and is shown schematically in Fig. 5:

- Assign an initial guess of the material parameters to an idealised vessel.
- Apply a displacement boundary condition to achieve the circumferential engineering strain experienced experimentally in an untreated artery at 100 mmHg at the outer diameter of the vessel (determined experimentally to be 20%, see Fig. 6).
- Iteratively optimise the maximum and minimum precrimp λ_p across the wall thickness of the undeformed configuration such that, under the displacement boundary condition (100 mmHg), the value of λ_f at every material point radially through the wall is approximately equal to 1.17, which is the fibre stretch value that has been previously determined to have the lowest fibre degradation rate (Gaul et al., 2018). It is our assumption that, in a healthy vessel, collagen will be at this stretch configuration (Fig. 5A).
- With the collagen precrimp λ_p set, apply an internal pressure to the vessel lumen and calibrate the material properties (E , k_1 , k_2) from experimental pressure-strain measurements (Fig. 6) using the optimised precrimp distribution, λ_p (Fig. 5B).

Fig. 6. Pressure-inflation curves for untreated porcine carotid arteries and the calibrated FE model. Individual vessels are shown in light grey with the average circumferential engineering strain for each pressure shown in black. The pressure-inflation response of the model is shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 5. Schematic showing A) calibration of the undeformed precrimp gradient only, by minimising the difference between the crimp occurring at a tissue strain equivalent to 100 mmHg and the crimp at which collagen is protected from degradation (approximately 17%). Once minimised, B) material properties are calibrated based on experimental pressure inflation curves. Calibration carried out in the absence of degradation.

2.4.3. Model calibration

The values obtained for collagen and matrix volume fraction ($\phi_f = 42\%$, $\phi_m = 58\%$) were determined previously via image processing (Gaul et al., 2018) and are supported by literature (de Figueiredo Borges et al., 2008; Fischer and Llauro, 1966; Tsamis et al., 2013). The gradient of precrimp, λ_p across the wall is insensitive to the choice of initial material parameters due to the use of a displacement-controlled boundary condition. The material properties of the vessel wall were calibrated from experimentally measured pressure diameter curves shown in Fig. 6 after the gradient of fibre precrimp, λ_p through the vessel wall was set (Section 2.4.2). Briefly, the Neo-Hookean non-collagenous matrix material stiffness, was calibrated from our experimental data at 60 mmHg where minimal collagen was judged to be contributing to the load. This assumption was based on our experimental pressure degradation observations and histological evaluation of low pressure samples, where minimal vessel expansion was observed as collagen was degraded (Fig. 7 and Fig. 8). Once the values for λ_p and the Neo-Hookean response were set, the anisotropic portion of our model was calibrated based on our experimental pressure-diameter curves by optimising k_1 and k_2 , to reduce the error between our experimental and computational pressure-diameter curves, as shown in Fig. 6. The parameters used in all simulations can be found in Table 1.

2.5. In-silico pressure inflation – degradation tests

To predict our pressure inflation-degradation experiments, we applied our degradation mechanics model to an idealised quarter finite element model of a cylindrical vessel geometry of similar dimensions to experimentally tested samples (inner radius $R_i = 2\text{mm}$, outer radius $R_o = 3\text{mm}$, length $l = 0.5\text{mm}$). Quadratic hexahedral hybrid elements with reduced integration were used to prevent element locking. Symmetry boundary conditions were applied to both circumferential side faces. The axial faces of the vessel were kept parallel using a constraint equation, mimicking the experimental setup described above and preventing convergence issues as the vessel degrades at different rates through its thickness. An internal luminal pressure was then applied and held constant to mimic the experimental setup. Degradation commenced only after full pressurisation of the vessel. Circumferential strain was exported and plotted against time during degradation for each pressure allowing for comparisons to be drawn with experimental data. The model was implemented using a user-defined material subroutine (UMAT) in the commercial finite element software, Abaqus 6.14 (Dassault Systèmes Simulia corporations, Vélizy-Villacoublay, France). A numerical method to compute the tangent matrix was implemented (Miehe, 1996; Nolan et al., 2014; Sun et al., 2008).

Fig. 8. Histological images of picrosirius red stained artery cross sections at 4x magnification for a A) control vessel and B) a vessel subjected to 60 mmHg in the presence of collagenase for 8 h showing complete collagen degradation (confirmed under polarised light by black image, not shown). Collagen shows as dark red. Despite complete collagen degradation, no significant vessel expansion is found in Fig. 7A for the samples under 60 mmHg pressure. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Parameters used in the current model.

Model component	Parameter	Value	Source
Vessel setup	ϕ_f	0.42	Gaul et al. (2018)
	ϕ_m	0.58	Gaul et al. (2018)
	λ_p^{min} (outer wall)	1.01	Calibrated
	λ_p^{max} (inner wall)	1.19	Calibrated
	R_i (mm)	2.00	Idealised
	R_o (mm)	3.00	Idealised
Material properties	l (mm)	0.5	Idealised
	E (MPa)	0.20	Calibrated
	ν	0.49	Idealised
	κ (MPa)	$\frac{E}{3(1-2\nu)}$	–
	G (MPa)	$\frac{E}{2(1+\nu)}$	–
	k_1 (kPa)	2.14	Calibrated
Collagen degradation	k_2	7.56	Calibrated
	$\theta_{collagen}$ (°)	0	Gaul et al. (2017)
	ϵ_{r1}^D	0.08	Gaul et al. (2018)
	ϵ_{r2}^D	0.23	Gaul et al. (2018)
	D_l (ms^{-1})	-1.50	Gaul et al. (2018)
	D_h (ms^{-1})	0.75	Gaul et al. (2018)
	D_c (ms^{-1})	0.21	Gaul et al. (2018)
	R	0.0001	Gaul et al. (2018)

Fig. 7. Experimental pressure-inflation response over time of A) vessels in purified bacterial collagenase ($n = 3$) and B) control vessels in PBS ($n = 1$). Noticeable creep can be observed in the degradation case, particularly at high pressures. Control vessels show minimal expansion after the initial creep response within the first 20 min. Individual vessels are plotted in grey while the mean response per pressure is shown in black alongside error bars of SD. Vessels which failed through rupture are marked with an x. Vessels which did not fail by rupture or last the 480-min test time failed due to the 16 ml syringe emptying because of increased vessel permeability.

3. Results

3.1. Model calibration

Experimental pressure-inflation experiments of the carotid vessels found a logarithmic type response with circumferential engineering strain plateauing at approximately 25% strain with increasing pressure (Fig. 6). A very close fit was achieved between our model and the mean experimental data with a root mean square error of 0.86% strain. As anticipated with biological tissue, however, considerable sample variability is observable with circumferential strain ranging from 19% to 33% at 180 mmHg.

3.2. Experimental degradation curves

Pressure inflation-degradation experiments showed significant circumferential vessel expansion with increasing pressure leading to vessel rupture (Fig. 7A). Vessels experiencing 180 mmHg showed the highest rate of expansion while negligible expansion was observed in vessels experiencing 60 mmHg despite complete collagen degradation (Fig. 8). Complete collagen degradation was confirmed by assessing polarised light images of picrosirius red stained histological tissue sections and noting the absence of any birefringence. Vessels which did not reach the 8 h end time of the experiment failed through either vessel rupture or loss of pressurisation fluid (collagenase) due to increasing vessel permeability during degradation. Vessels which failed through rupture are indicated symbolically in Fig. 7A.

Whilst a prescribed axial tethering was not imposed on the vessels during these experiments, the axial strain experienced by the vessels during pressurisation was measured using the DIC system and is reported in Supplementary data Fig. 1.3.

Significant reductions in vessel thickness (0.919 ± 0.026 (0.112) mm vs. 0.673 ± 0.031 (0.133) mm, $p < 0.0001$) were also observed using a measuring microscope post degradation using a paired Wilcoxon nonparametric test for a non-normal frequency distribution (D'Agostino-Pearson normality test). Results are expressed as mean $\pm$ SEM (SD).

Experimental pressure inflation experiments for undegraded control vessels (Fig. 7B) show no creep occurring with increasing time in comparison to collagenase treated vessels. Control vessels were found to reach approximately 90% of their final circumferential engineering strain within 20 min of pressurisation. No vessel failure was observed.

3.3. Investigation of low pressure degradation

Vessels experiencing 100 mmHg prior to pressurising to 180 mmHg exhibited significantly longer ($p < 0.05$) failure times compared to those experiencing an initial 60 mmHg. This longer failure time suggests a strain-induced protection mechanism occurs at 100 mmHg (Fig. 9). Failure time analysis provides additional insight, which is not visible in Fig. 7. One outlier as determined through a Grubbs outlier test ($\alpha =$

Fig. 9. Time to failure for specimen experiencing 180 mmHg only (Fig. 7A) as well as those which were initially pressurised to 60 mmHg or 100 mmHg for 45 min before a high pressure of 180 mmHg was applied until vessel failure ($n = 4$). Vessels initially experiencing 100 mmHg show increased time to failure suggesting slower initial degradation i.e. protection.

0.05) was removed from the 100/180 mmHg data set, although this did not affect statistical significance. Complete data set including the outlier measurement is shown in Supplementary material.

3.4. Modelling of collagen degradation in arteries

A similar degradation expansion response was predicted by our model, with the highest rate of vessel expansion occurring at 180 mmHg (Fig. 10). Our model also found minimal vessel expansion occurring at 60 mmHg as observed experimentally, despite a high rate of collagen degradation. Differences can be seen between the rate of expansion predicted by our model and that observed experimentally, which may arise due to unaccounted factors such as the influence of diffusion of bacterial collagenase at different experimental pressures. At high pressure (140 mmHg and 180 mmHg), as collagen content reduces, strain exponentially increases, and the quasi-static simulation no longer converges upon a solution. Consequently, the simulation is stopped at 42% circumferential strain. The 100 mmHg condition can be observed to level off at approximately 33% circumferential strain. At this point the collagen volume fraction has reduced to 0% and the intraluminal pressure is borne by the non-collagenous matrix alone. Plots of the collagen fibre volume fraction ϕ_f and fibre stretch λ_f are shown for 100 mmHg in Fig. 11.

4. Discussion

Degradation of structurally significant collagen fibres is a critical process in healthy arterial remodelling; however, excessive degradation could potentially compromise vessel integrity leading to development of progressive, degenerative diseases such as aneurysm formation. The objective of this study was to investigate the degradation response of intact arterial vessels at physiologically relevant pressures using a combination of experimental and computational approaches.

In collagenase treated vessels, increasing vessel pressure resulted in an increased initial circumferential strain, an increased rate of vessel expansion and a reduced time to failure (Fig. 7). Ostensibly, these findings bear similarities to previous creep studies on collagenase treated bovine pericardium which found a reduced time to failure with increasing load (Ellsmere et al., 1999). Unlike our findings, a higher initial rate of expansion is observed in bovine pericardium experiencing low loads. These differences can be attributed to the greater mechanical contribution of non-collagenous matrix in arterial tissue which is the dominant load bearing constituent at low pressures (Roach and Burton, 1957), an effect which was found to be dominant in the degradation mechanics in our previous study (Gaul et al., 2018). This matrix response masks the underlying degradation response of collagen, especially at low pressures. Consequently, our low pressure condition (60

Fig. 10. Computational pressure-inflation response of degrading vessels at 60, 100, 140 and 180 mmHg. Noticeable vessel expansion can be observed as vessels degrade, particularly at high pressures.

Fig. 11. A) collagen content and B) collagen fibre stretch are shown at i) 0 mmHg and ii) 100 mmHg prior to degradation and iii) 100 mmHg after 1500 min of degradation. At 100 mmHg before degradation, the gradient of collagen fibre stretch is minimised about the 17% protection strain. As the vessel degrades, vessel stretch and consequently fibre stretch increases leading to further degradation. Note change of legend for collagen stretch at 100 mmHg.

mmHg), which was assumed to have a high rate of collagen degradation (Gaul et al., 2018), exhibited minimal degradation induced expansion. Consequently, there was no tissue level failure. Nevertheless, histological analysis of these vessels confirmed complete collagen degradation (see Fig. 8). This suggests that non-collagenous matrix prevents further vessel expansion at low pressures even in the absence of collagen. This hypothesis is supported by Fig. 6, which shows a linear strain-pressure response up to 80 mmHg, suggesting that collagen doesn't contribute significantly up to this pressure.

The increased rate of degradation at low pressures was also demonstrated through the two-pressure failure tests in Fig. 9 where one group was held at 60 mmHg and another at 100 mmHg for 45 min before being pressurised to 180 mmHg. The shorter time to failure in the 60–180 mmHg group compared to the 100–180 mmHg group suggests that a higher degradation rate occurred at 60 mmHg when compared to 100 mmHg. Extending these findings to *in vivo* conditions suggests that, although degradation may be elevated in low pressure environments, tissue failure is unlikely unless high pressure and subsequent increased strain conditions develop. This response could help to explain the increased risk of adverse clinical cardiovascular events associated with hypertension (WHO, 2014).

Our complementary computational model, calibrated from experimental data independent of the full artery pressure-expansion results presented in this paper, predicted a qualitatively similar degradation response occurring at each pressure (Fig. 7). The model supported experimental observations that minimal vessel expansion occurs with collagen degradation at 60 mmHg due to the dominant mechanical contribution of the non-collagenous matrix. A similar response is also observed in our 100 mmHg model where strain plateaus as collagen is removed and pressure is borne by non-collagenous matrix alone. This response is partially observed experimentally, with the two 100 mmHg tests found not to fail by rupture also displaying a plateauing at approximately 200 min. We previously reported a similar non-collagenous matrix influence occurring in flat dogbone specimens of arterial tissue which masked an underlying V-shaped collagen degradation response (Gaul et al., 2018). Despite the same apparent mechanism being responsible for the current findings, degradation manifests itself differently during pressure dependent testing (increasing expansion rate with increasing pressure), which may lead one to incorrectly conclude that degradation linearly increases with pressure or strain. The

model also supports the hypothesis that a degradation minimum exists at healthy physiological pressures (100 mmHg), which may be the body's way of maintaining homeostasis or equilibrium *in vivo*. Consequently, abnormal blood pressure levels may lead to accelerated collagen degradation which could exceed collagen production. Interestingly, a number of studies on other biological tissues have also found a V-shaped degradation response occurring at the tissue level (Ghazanfari et al., 2016a; Yi et al., 2016). This observation suggests that for these tissues, i.e. pericardium and lung respectively, the non-collagenous matrix has a reduced mechanical contribution at low strains which does not mask the underlying collagen degradation response.

Although the same trend in degradation was found between our model and the experimental results, the rate at which degradation occurs was found to differ significantly. These differences are likely influenced by several factors. Firstly, although there is no significant creep observed in control vessels, there may be long-term pressure-dependent creep mechanisms influencing the degradation results. The rate of collagenase diffusion through the artery wall also differs at each pressure condition, with greater diffusion occurring at higher pressures (Zareian et al., 2010). There is little or no pressure induced diffusion in the experiments from which some of the computational model parameters are derived (Gaul et al., 2018). The influence of diffusion of bacterial collagenase is also highlighted histologically when comparing the timeframes required to investigate degradation with and without pressure (see Supplementary material). As a result, the rate of degradation and subsequent vessel expansion identified computationally is much slower than that observed experimentally. Secondly, sample variability, which was noted in Fig. 6, is likely to influence the fibre strain, and consequently, the rate of collagen degradation. Additional failure modes other than fibre degradation such as fibre rupture are also likely to occur at increased pressures and may lead to further differences between experimental and computational results.

A number of computational models have been developed to investigate collagen remodelling in soft tissues adopting strain- and energy-based approaches to reduce collagen volume fraction or collagen fibre radius. However, many of these models assume a strain-protection mechanism only in the prediction of tissue degradation *ex vivo* (Gyoneva et al., 2016; Hadi et al., 2012; Heck et al., 2015; Tonge et al., 2015). Similar degradation models have been employed to investigate valve

retraction in tissue-engineered heart valves (Loerakker et al., 2016) and lamina cribosa thickening in glaucoma (Grytz et al., 2012) *in vivo*; however, to our knowledge, this is the first time that such a computational approach, informed by tailored experiments, has been adopted for investigating potential degenerative arterial diseases.

The proposed computational model may now provide insights into fatal degenerative arterial diseases such as aneurysm progression or arterial disease within complex vessel geometries and loading environments. Applying this model to arterial diseases such as atherosclerosis may help identify vessels at risk of plaque rupture due to excessive collagen degradation at sites of higher or low strain. Similarly, the current model may also aid in our understanding of the remodelling response caused by medical devices such as stents and prosthetic heart valves, which change the local mechanical environment of arterial collagen (Ghazanfari et al., 2016b).

A limitation of the current study is the use of bacterial collagenase rather than physiologically relevant MMPs and cysteine collagenases, which degrade collagen *in vivo* (Abdul-Hussien et al., 2007; Aikawa, 2004). Bacterial collagenases also commonly contain non-specific enzymes which can influence the experimental degradation response; however, this was mitigated through the use of purified bacterial collagenase (Gaul et al., 2018). Diffusion of the collagenase solution through the vessel wall is also likely to influence the rate of degradation observed (Zareian et al., 2010), with accelerated diffusion occurring at higher pressures. Unfortunately, diffusion and pressure are intrinsically linked in the current experimental setup, with pressure dictating the strain experienced in the vessel wall. Computationally, a diffusion rate could be incorporated to account for these differences, although the current predictions are suitably accurate even without accounting for diffusion. Furthermore, diffusion would only serve to increase the accuracy of our model in predicting our experimental data, given that diffusion is less physiologically relevant when one considers that *in vivo* collagenase production occurs *in situ* (Aikawa, 2004). Vessel variability is also likely to influence the degradation response based on factors such as vessel diameter and tissue thickness as well as collagen and elastin content. The current experimental setup is also designed to neglect axial tethering, in order to determine a clear understanding of how the degradation response differs in intact vessels and uniaxial dogbone specimens (Gaul et al., 2018). Interestingly, Ellsmere et al. found that cyclic loading, an environment experienced physiologically by arterial tissue, accelerated the degradation response of bovine pericardium. Future work will consider these more complex loading conditions and the axial stretch on the vessel which would likely have a significant influence on the collagen fibre strains.

The results of the current study have identified, for the first time, the strain-dependent degradation response of intact arterial vessels at physiologically relevant pressures. These findings have significantly expanded on previous work on flat dogbone arterial specimens, identifying a different tissue level response occurring in the native arterial state. Increased rates of vessel expansion were found to occur at high intraluminal pressures, along with an increased rate of collagen degradation at low vessel pressures. These findings highlight how arterial collagen strain may contribute to accelerated collagen degradation in diseases such as aneurysm progression *in vivo* and may also apply in other progressive degenerative vascular diseases, including the biological response to intravascular medical devices such as stents.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

- Abdul-Hussien, H., Soekhoe, R.G.V., Weber, E., von der Thüsen, J.H., Kleemann, R., Mulder, A., van Bockel, J.H., Hanemaaijer, R., Lindeman, J.H.N., 2007. Collagen degradation in the abdominal aneurysm. *Am. J. Pathol.* 170, 809–817. <https://doi.org/10.2353/ajpath.2007.060522>.
- Aikawa, M., 2004. Vascular biology of collagenases in vulnerable atherosclerotic plaques. *Int. Congr. Ser.* 1262, 67–70. <https://doi.org/10.1016/j.ics.2003.12.056>.
- Bersi, M.R., Bellini, C., Humphrey, J.D., Avril, S., 2019. Local variations in material and structural properties characterize murine thoracic aortic aneurysm mechanics. *Biomech. Model. Mechanobiol.* 18 (1), 203–218. <https://doi.org/10.1007/s10237-018-1077-9>.
- Chang, S.-W., Buehler, M.J., 2014. Molecular biomechanics of collagen molecules. *Mater. Today* 17, 70–76. <https://doi.org/10.1016/j.mattod.2014.01.019>.
- Chang, S.-W., Flynn, B.P., Ruberti, J.W., Buehler, M.J., 2012. Molecular mechanism of force induced stabilization of collagen against enzymatic breakdown. *Biomaterials* 33, 3852–3859. <https://doi.org/10.1016/j.biomaterials.2012.02.001>.
- Daemen, M.J., Ferguson, M.S., Gijzen, F.J., Hippe, D.S., Kooi, M.E., Demarco, K., van der Wal, A.C., Yuan, C., Hatsukami, T.S., 2016. Carotid plaque fissure: an underestimated source of intraplaque hemorrhage. *Atherosclerosis* 254, 102–108. <https://doi.org/10.1016/j.atherosclerosis.2016.09.069>.
- de Figueiredo Borges, L., Jaldin, R.G., Dias, R.R., Stolf, N.A.G., Michel, J.B., Gutierrez, P. S., 2008. Collagen is reduced and disrupted in human aneurysms and dissections of ascending aorta. *Hum. Pathol.* 39, 437–443. <https://doi.org/10.1016/j.humpath.2007.08.003>.
- Driessen, N.J.B., Mol, A., Bouten, C.V.C., Baaijens, F.P.T., 2007. Modeling the mechanics of tissue-engineered human heart valve leaflets. *J. Biomech.* 40, 325–334. <https://doi.org/10.1016/j.jbiomech.2006.01.009>.
- Ellsmere, J.C., Khanna, R.A., Michael Lee, J., 1999. Mechanical loading of bovine pericardium accelerates enzymatic degradation. *Biomaterials* 20, 1143–1150. [https://doi.org/10.1016/S0142-9612\(99\)00013-7](https://doi.org/10.1016/S0142-9612(99)00013-7).
- Fischer, G.M., Llauro, J.G., 1966. Collagen and elastin content in canine arteries selected from functionally different vascular beds. *Circ. Res.* 19, 394–399. <https://doi.org/10.1161/01.RES.19.2.394>.
- Gaul, R.T., Nolan, D.R., Lally, C., 2017. Collagen fibre characterisation in arterial tissue under load using SALS. *J. Mech. Behav. Biomed. Mater.* 75, 359–368. <https://doi.org/10.1016/j.jmbbm.2017.07.036>.
- Gaul, R.T., Nolan, D.R., Ristori, T., Bouten, C.V.C., Loerakker, S., Lally, C., 2018. Strain mediated enzymatic degradation of arterial tissue: insights into the role of the non-collagenous tissue matrix and collagen crimp. *Acta Biomater.* 77, 301–310. <https://doi.org/10.1016/j.actbio.2018.06.037>.
- Ghazanfari, S., Driessen-Mol, A., Bouten, C.V.C., Baaijens, F.P.T., 2016a. Modulation of collagen fiber orientation by strain-controlled enzymatic degradation. *Acta Biomater.* 35, 118–126. <https://doi.org/10.1016/j.actbio.2016.02.033>.
- Ghazanfari, S., Driessen-Mol, A., Hoerstrup, S.P., Baaijens, F.P.T., Bouten, C.V.C., 2016b. Collagen matrix remodeling in stented pulmonary arteries after transcatheter heart valve replacement. *Cells Tissues Organs* 201, 159–169. <https://doi.org/10.1159/000442521>.
- Grytz, R., Sigal, I.A., Ruberti, J.W., Meschke, G., Crawford Downs, J., 2012. Lamina cribrosa thickening in early glaucoma predicted by a microstructure motivated growth and remodeling approach. *Mech. Mater.* 44, 99–109. <https://doi.org/10.1016/j.mechmat.2011.07.004>.
- Gyoneva, L., Hovell, C.B., Pewowaruk, R.J., Dorfman, K.D., Segal, Y., Barocas, V.H., 2016. Cell–matrix interaction during strain-dependent remodelling of simulated collagen networks. *Interface Focus* 6. <https://doi.org/10.1098/rsfs.2015.0069>, 20150069.
- Hadi, M.F., Sander, E.A., Ruberti, J.W., Barocas, V.H., 2012. Simulated remodeling of loaded collagen networks via strain-dependent enzymatic degradation and constant-rate fiber growth. *Mech. Mater.* 44, 72–82. <https://doi.org/10.1016/j.mechmat.2011.07.003>.
- Heck, T.A.M., Wilson, W., Foolen, J., Cilgindir, A.C., Ito, K., van Donkelaar, C.C., 2015. A tissue adaptation model based on strain-dependent collagen degradation and contact-guided cell traction. *J. Biomech.* 48, 823–831. <https://doi.org/10.1016/j.jbiomech.2014.12.023>.
- Holzappel, G.A., Sommer, G., Auer, M., Regitnig, P., Ogden, R.W., 2007. Layer-specific 3D residual deformations of human aortas with non-atherosclerotic intimal thickening. *Ann. Biomed. Eng.* 35, 530–545. <https://doi.org/10.1007/s10439-006-9252-z>.
- Huang, C., Yannas, I.V., 1977. Mechanochemical studies of enzymatic degradation of insoluble collagen fibers. *J. Biomed. Mater. Res.* 11, 137–154. <https://doi.org/10.1002/jbm.820110113>.

- Junqueira, L.C.U., Bignolas, G., Brentani, R.R., 1979. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem. J.* 11, 447–455. <https://doi.org/10.1007/BF01002772>.
- Krasny, W., Morin, C., Magoaric, H., Avril, S., 2017. A comprehensive study of layer-specific morphological changes in the microstructure of carotid arteries under uniaxial load. *Acta Biomater.* 57, 342–351. <https://doi.org/10.1016/j.actbio.2017.04.033>.
- Loerakker, S., Obbink-Huizer, C., Baaijens, F.P.T., 2014. A physically motivated constitutive model for cell-mediated compaction and collagen remodeling in soft tissues. *Biomech. Model. Mechanobiol.* 13, 985–1001. <https://doi.org/10.1007/s10237-013-0549-1>.
- Loerakker, S., Ristori, T., Baaijens, F.P.T., 2016. A computational analysis of cell-mediated compaction and collagen remodeling in tissue-engineered heart valves. *J. Mech. Behav. Biomed. Mater.* 58, 173–187. <https://doi.org/10.1016/j.jmbbm.2015.10.001>.
- Lotz, J.C., Hadi, T., Bratton, C., Reiser, K.M., Hsieh, A.H., 2008. Anulus fibrosus tension inhibits degenerative structural changes in lamellar collagen. *Eur. Spine J.* 17, 1149–1159. <https://doi.org/10.1007/s00586-008-0721-y>.
- Miehe, C., 1996. Numerical computation of algorithmic (consistent) tangent moduli in large-strain computational inelasticity. *Comput. Methods Appl. Mech. Eng.* 134, 223–240. [https://doi.org/10.1016/0045-7825\(96\)01019-5](https://doi.org/10.1016/0045-7825(96)01019-5).
- Miles, C.A., Ghelashvili, M., 1999. Polymer-in-a-Box mechanism for the thermal stabilization of collagen molecules in fibers. *Biophys. J.* 76, 3243–3252. [https://doi.org/10.1016/S0006-3495\(99\)77476-X](https://doi.org/10.1016/S0006-3495(99)77476-X).
- Müller-Schweinitzer, E., 2009. Cryopreservation of vascular tissues. *Organogenesis* 5, 97–104. <https://doi.org/10.4161/org.5.3.9495>.
- Nabeshima, Y., Grood, E.S., Sakurai, A., Herman, J.H., 1996. Uniaxial tension inhibits tendon collagen degradation by collagenase in vitro. *J. Orthop. Res.* 14, 123–130. <https://doi.org/10.1002/jor.1100140120>.
- Nolan, D.R., Gower, a.L., Destrade, M., Ogden, R.W., McGarry, J.P., 2014. A robust anisotropic hyperelastic formulation for the modelling of soft tissue. *J. Mech. Behav. Biomed. Mater.* 39, 48–60. <https://doi.org/10.1016/j.jmbbm.2014.06.016>.
- O'Connor, C.A., Nolan, D.R., McEvoy, E., McGarry, J.P., 2017. A three part hyperelastic law for anisotropic aortic tissue: model development and experimental validation. In: *Summer Biomechanics, Bioengineering and Biotransport Conference*. Arizona.
- Pegg, D.E., Wusteman, M.C., Boylan, S., 1997. Fractures in cryopreserved elastic arteries. *Cryobiology* 34, 183–192. <https://doi.org/10.1006/cryo.1996.1997>.
- Roach, M.R., Burton, a C., 1957. The reason for the shape of the distensibility curves of arteries. *Can. J. Biochem. Physiol.* 35, 681–690. <https://doi.org/10.1139/o57-080>.
- Robitaille, M.C., Zareian, R., Dimarzio, C. a, Wan, K.-T., Ruberti, J.W., 2011. Small-angle light scattering to detect strain-directed collagen degradation in native tissue. *Interface Focus* 1, 767–776. <https://doi.org/10.1098/rsfs.2011.0039>.
- Ruberti, J.W., Hallab, N.J., 2005. Strain-controlled enzymatic cleavage of collagen in loaded matrix. *Biochem. Biophys. Res. Commun.* 336, 483–489. <https://doi.org/10.1016/j.bbrc.2005.08.128>.
- Sáez, P., García, A., Peña, E., Gasser, T.C., Martínez, M.A., 2016. Microstructural quantification of collagen fiber orientations and its integration in constitutive modeling of the porcine carotid artery. *Acta Biomater.* 33, 183–193. <https://doi.org/10.1016/j.actbio.2016.01.030>.
- Schneider, C. a, Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Schriebl, A.J., Zeindlinger, G., Pierce, D.M., Regitnig, P., Holzapfel, G.A., 2012. Determination of the layer-specific distributed collagen fibre orientations in human thoracic and abdominal aortas and common iliac arteries. *J. R. Soc. Interface* 9, 1275–1286. <https://doi.org/10.1098/rsif.2011.0727>.
- Shahid, S.S., Gaul, R.T., Kerskens, C.M., Flamini, V., Lally, C., 2017. Quantifying the ultrastructure of carotid artery using high-resolution micro-diffusion tensor imaging – comparison of intact vs. open cut tissue. *Phys. Med. Biol.* 62, 8850–8868. <https://doi.org/10.1088/1361-6560/aa9159>.
- Sun, W., Chaikof, E.L., Levenston, M.E., 2008. Numerical approximation of tangent moduli for finite element implementations of nonlinear hyperelastic material models. *J. Biomech. Eng.* 130, 061003. <https://doi.org/10.1115/1.2979872>.
- Thompson, R.W., Geraghty, P.J., Lee, J.K., 2002. Abdominal aortic aneurysms: basic mechanisms and clinical implications. *Curr. Probl. Surg.* 39, 110–230. <https://doi.org/10.1067/msg.2002.121421>.
- Tonge, T.K., Ruberti, J.W., Nguyen, T.D., 2015. Micromechanical modeling study of mechanical inhibition of enzymatic degradation of collagen tissues. *Biophys. J.* 109, 2689–2700. <https://doi.org/10.1016/j.bpj.2015.10.051>.
- Trabelsi, O., Dumas, V., Breyse, E., Laroche, N., Avril, S., 2019. In vitro histomechanical effects of enzymatic degradation in carotid arteries during inflation tests with pulsatile loading. *J. Mech. Behav. Biochem. Mater.* 103, 103550.
- Tsamis, A., Krawiec, J.T., Vorp, D. a, 2013. Elastin and collagen fibre microstructure of the human aorta in ageing and disease: a review. *J. R. Soc. Interface* 10. <https://doi.org/10.1098/rsif.2012.1004>, 20121004.
- WHO, 2014. *Global Status Report on Noncommunicable Diseases 2014*.
- Willett, T.L., Labow, R.S., Lee, J.M., 2008. Mechanical overload decreases the thermal stability of collagen in an in vitro tensile overload tendon model. *J. Orthop. Res.* 26, 1605–1610. <https://doi.org/10.1002/jor.20672>.
- Wyatt, K.E.-K., Bourne, J.W., Torzilli, P. a, 2009. Deformation-dependent enzyme mechanokinetic cleavage of type I collagen. *J. Biomech. Eng.* 131, 051004. <https://doi.org/10.1115/1.3078177>.
- Yi, E., Sato, S., Takahashi, A., Parameswaran, H., Blute, T.A., Bartolák-Suki, E., Suki, B., 2016. Mechanical forces accelerate collagen digestion by bacterial collagenase in lung tissue strips. *Front. Physiol.* 7, 1–12. <https://doi.org/10.3389/fphys.2016.00287>.
- Zareian, R., Church, K.P., Saeidi, N., Flynn, B.P., Beale, J.W., Ruberti, J.W., 2010. Probing collagen/enzyme mechanochemistry in native tissue with dynamic, enzyme-induced creep. *Langmuir* 26, 9917–9926. <https://doi.org/10.1021/la100384e>.