Strain mediated enzymatic degradation of arterial tissue

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Full length article

Strain mediated enzymatic degradation of arterial tissue: Insights into the role of the non-collagenous tissue matrix and collagen crimp

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ABSTRACT

Collagen fibre remodelling is a strain dependent process which is stimulated by the degradation of existing collagen. To date, literature has focussed on strain dependent degradation of pure collagen or structurally simple collagenous tissues, often overlooking degradation within more complex, heterogeneous soft tissues. The aim of this study is to identify, for the first time, the strain dependent degradation behaviour and mechanical factors influencing collagen degradation in arterial tissue using a combined experimental and numerical approach.

To achieve this, structural analysis was carried out using small angle light scattering to determine the fibre level response due to strain induced degradation. Next, strain dependent degradation rates were determined from stress relaxation experiments in the presence of crude and purified collagenase to determine the tissue level degradation response. Finally, a 1D theoretical model was developed, incorporating matrix stiffness and a gradient of collagen fibre crimp to decouple the mechanism behind strain dependent arterial degradation.

SALS structural analysis identified a strain mediated degradation response in arterial tissue at the fibre level not dissimilar to that found in literature for pure collagen. Interestingly, two distinctly different strain mediated degradation responses were identified experimentally at the tissue level, not seen in other collagenous tissues. Our model was able to accurately predict these experimental findings, but only once the load bearing matrix, its degradation response and the gradient of collagen fibre crimp across the arterial wall were incorporated. These findings highlight the critical role that the various tissue constituents play in the degradation response of arterial tissue.

Statement of Significance

Collagen fibre architecture is the dominant load bearing component of arterial tissue. Remodelling of this architecture is a strain dependent process stimulated by the degradation of existing collagen. Despite this, degradation of arterial tissue and in particular, arterial collagen, is not fully understood or studied. In the current study, we identified for the first time, the strain dependent degradation response of arterial tissue, which has not been observed in other collagenous tissues in literature. We hypothesised that this unique degradation response was due to the complex structure observed in arterial tissue. Based on this hypothesis, we developed a novel numerical model capable of explaining this unique degradation response which may provide critical insights into disease development and aid in the design of interventional medical devices.

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1. Introduction

Collagen fibre architecture is critical in healthy arterial function, and it is believed that maladaptive remodelling of this fibrous architecture may be involved in the development and progression

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of arterial disease [1]. Such remodelling may also play a critical role in the biological response to intravascular devices, such as stents, that dramatically alter the mechanical environment within an artery [2–4]. To date, literature has predominantly considered strain induced fibre reorientation [5–8] and there is a sparsity of information available on the production and degradation of collagen that occurs alongside fibre reorientation, both *in vivo* and *in vitro*. Further investigation of these remodelling processes could help our understanding of the role strain plays in the degeneration of arterial tissue in disease [6,9,10] and following medical device intervention which can alter the mechanical environment of the vessel [11,12].

Previous studies on other soft tissues, such as tendon, corneal tissue and bovine pericardium, have shown apparently conflicting results on whether strain inhibits [13–18] or enhances [19,20] enzymatic collagen degradation. In the stress relaxation study of individual collagen fibres obtained from rat tail tendon, Wyatt et al. [13] found a 73% decrease in enzymatic activity as relaxation strain increased from 1% to 4%. This finding was supported by fibre diameter measurements pre- and post-collagenase treatment. Similarly, Robitaille et al. [15] investigated the role of low strain conditions in the degradation of corneal collagen fibres in the presence of bacterial collagenase. Using small angle light scattering (SALS), they found that the application of a 6% tissue strain increased fibre alignment with time as compared to unstrained tissue, suggesting a protective mechanism for strained fibres. In contrast, Ellsmere et al. [19] found an increase in the rate of enzymatic cleavage in bovine pericardium in response to increasing loads of 1 g, 10 g and 60 g. This increase was identified by a reduced time to failure in creep tests as the initial load was increased. Interestingly, this trend was more pronounced when collagen was subject to dynamic loading conditions, an environment experienced by arteries *in vivo*. It is worth noting however, that higher creep rates and thus accelerated degradation may have occurred initially at low loads based on the results presented.

These different degradation responses may be explained by a study by Huang and Yannas [21] on reconstituted collagen in the form of collagen tapes. Huang and Yannas experimentally measured the level of enzymatic collagen degradation of these tapes at known strains, ranging from 1 to 7%. It was concluded that accelerated collagen degradation occurred at both high and low strain levels, while, an intermediary, strain induced protective region was found close to 4% strain. More recently, Ghazanfari et al. [22] also identified a similar strain dependent degradation response in fresh decellularised bovine pericardium. Compared to Huang and Yannas [21], in Ghazanfari et al. a marked increase in the tissue strain at which degradation is at minimum was identified, which may be explained by the initial reorientation and straightening of constituent mature fibres with applied strain. Whilst these previous studies have investigated the strain- and load-mediated degradation of collagen fibres in structurally simple collagenous tissues, they have overlooked collagen in its native state within more complex, organised soft tissues.

Arterial tissue, in contrast to pure collagen or bovine pericardium, can be considered as structurally more heterogeneous with circumferentially orientated collagen fibres [8] embedded in a complex network of cells and other extracellular material. In arterial tissue, matrix stiffness and collagen fibre crimp, present through the wall thickness [23–25], may play a significant role in collagen degradation and tissue remodelling but this has yet to be determined. Furthermore, previous studies have only focussed on small strain ranges, potentially missing critical information across larger strain ranges which may be the case in existing studies of collagenous tissues [2,3,15]. These strain environments may manifest themselves in arterial tissue through atherosclerosis induced stiffening, aneurysm development and vessel expansion

due to stenting. It has yet to be established which, if any, of these strain dependent degradation profiles exist in arterial tissue. If established, these responses may play a pivotal role in our understanding of the development, progression and treatment of diseased tissue.

Given the complexity and structural heterogeneity of the arterial tissue, fully understanding the mechanisms determining the effects of strain on collagen degradation is extremely difficult. Mathematical and computational models can help to unravel these mechanisms, due to their versatility and the potential to isolate single parameters. A number of numerical studies have been developed to explore remodelling of arterial tissue [1,26] and other soft collagenous tissues [27–31], and many of these include explicit formulations for the degradation of collagen [27–30]. Although these computational models are based on experimental data: [29,30] using [19], [30] using [17]; and [28] using [21], no model to-date has compared *in silico* predictions, incorporating collagen degradation, to experimentally obtained strain- or load-induced degradation of intact arterial tissue.

The overall objective of this study was to determine the strain-dependent degradation response of arterial tissue, and in particular, that of the load-bearing collagen fibres within the tissue. To achieve this, the study had the following specific aims; 1) to directly investigate changes in collagen fibre distributions in loaded tissues enzymatically treated with collagenase, 2) to investigate the degradation rate of arterial tissue *ex vivo* subject to increasing strains through a series of uniaxial stress relaxation experiments, in the presence of crude and purified bacterial collagenase, and 3) to develop a theoretical model of arterial degradation to elucidate the influence of non-collagenous matrix and collagen crimp in the overall degradation response of the arterial tissue.

2. Tissue preparation

Fresh porcine common carotid arteries were excised from 6-month-old Large White pigs ($n = 38$) weighing approximately 80 kg at the time of slaughter. Carotid arteries were transported on ice and frozen to -80°C at a controlled rate of $-1^{\circ}\text{C}/\text{min}$ in the presence of a cryoprotectant to maintain mechanical and structural properties [32]. Samples were preserved for a period of 2–3 weeks prior to mechanical and structural analysis of the tissue. Upon thawing in a water bath at 37°C [33], vessels were cut longitudinally and opened flat removing residual strains (Fig. 1A) before circumferential (crude: $n = 13$; purified: $n = 16$) and axial (crude: $n = 13$) dogbone specimens were cut for mechanical analysis. Crude collagenase contains a mixture of collagenase and other non-specific proteases which results in the degradation of collagenous and non-collagenous tissue (Fig. 1B). In contrast, purified collagenase has been treated to contain minimal secondary proteolytic activity, allowing for more selective collagen degradation (Fig. 1B) [34]. Circumferential strips (crude: $n = 17$) of width 4 mm were also cut for structural analysis using SALS. Arterial strips were required for SALS assessment due to their larger surface area, which reduced after collagenase treatment. The intima and adventitia were then carefully removed as carried out elsewhere [8,35], to ensure a consistent circumferential collagen fibre architecture [8] and to focus on the most mechanically relevant, medial layer within the vessel wall [35]. Removing the intima and adventitia also allowed transmission of laser light through the sample, a prerequisite of SALS analysis. Sample thickness after layer separation was recorded using a measuring microscope with a micrometer controlled stage for dogbone specimens: 0.711 ± 0.017 (0.112) mm, and strip specimens: 0.646 ± 0.056 (0.144) mm. Dimensions are expressed as mean $\pm$ SEM (SD).

Fig. 1. A) Schematic showing gradient of collagen fibre crimp which is exaggerated once residual strains are removed and B) histological images of picrosirius red stained artery cross sections showing matrix and collagen degradation in the presence of crude collagenase in comparison to selective collagen degradation in the presence of purified collagenase. Collagen shows as dark red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Structural response

3.1. SALS analysis

An in-house SALS system was used to assess changes in fibre architecture due to strain dependent degradation. The system consists of an unpolarised 5 mW HeNe laser ($\lambda = 632.8$ nm; JDSU, Newbury, UK), focussing lens ($f_1 = 75$ mm; Edmund Optics Ltd, York, UK), automated sample positioner, projection screen and a CMOS USB camera as described elsewhere [8]. In SALS, laser light is passed through a fibrous test sample and scattered orthogonally to the central axis of the samples constituent fibres. From this scattered light pattern, orientation and alignment information may be determined.

SALS structural analysis was carried out on circumferential strip specimens which were uniaxially strained and placed in crude bacterial collagenase at 37 °C for 4 h. Specimens were analysed by SALS before and after crude collagenase treatment to identify relative changes in fibre alignment (Fig. 2A). Specimens were interrogated over a 2 mm $\times$ 1.5 mm region at the centre of each sample at 192 interrogation regions using a 150 μ m beam diameter and a 125 μ m scanning resolution.

The scattered light distribution recorded using SALS was used to identify relative changes in fibre eccentricity, E , a measure of fibre distribution. Eccentricity is determined by the ratio of major to minor axis of the scattered light ellipse shown in Fig. 2B using Eq. (1). An eccentricity of 1 represents perfect fibre alignment in

one direction while an eccentricity of 0 corresponds to an isotropic distribution of fibres. Eccentricity results across the 192 interrogation regions were averaged for each sample.

$$E = \frac{\sqrt{r_{maj}^2 - r_{min}^2}}{r_{maj}} \quad (1)$$

where r_{maj} and r_{min} are the major and minor axis of the scattered light ellipse respectively.

3.2. Results: SALS fibre analysis

SALS analysis of arterial strips subjected to crude collagenase showed strain dependent structural changes (Fig. 3). High relative increases in eccentricity, representing alignment, can be seen at low strain levels (<5%), before a sharp reduction in eccentricity is observed with increasing strain level (>5%). Eccentricity is next seen to plateau (>10%) before a large drop off at high strain levels (>40%).

4. Mechanical testing of enzymatic digested tissue

4.1. Experimental setup

To assess the tissue level mechanical response to collagen degradation, uniaxial stress relaxation tests were also carried out on circumferential and axial dogbone specimens to determine

Fig. 2. A) Flow diagram showing SALS analysis to determine relative changes in eccentricity and B) SALS images showing a high ratio (high alignment) and low ratio (low alignment) of major to minor axis.

Fig. 3. Relative change in eccentricity for circumferential arterial strip samples following 4 h held at a constant nominal strain in crude bacterial collagenase for a range of strains. $n = 17$.

tissue and collagen degradation rates using a Zwick/Roell Z005 material testing machine (Zwick/Roell, Germany) with a 20N load cell. Each specimen was instantaneously strained to a predefined strain level and held at this strain level in a PID temperature controlled PBS bath at 37 °C (Fig. 4A). The specimens were allowed to relax for 60 min before the PBS was replaced with an equal volume of either crude (Type I, 87.5 U/ml; Sigma-Aldrich, Ireland) or purified (400 U/ml; Worthington Biochemical, USA) bacterial collagenase in PBS supplemented with calcium and magnesium (D8662; Sigma-Aldrich, Ireland). The force decay due to degradation was monitored for a further 3 h for samples treated with crude collagenase and 7 h for those treated with the slower acting purified collagenase, resulting in a total test time of 4 h and 8 h respectively (Fig. 4B). Purified collagenase was used to more selectively digest collagen, allowing for the isolation of the collagen degradation response from that of collagen and matrix degradation which was observed with crude collagenase (Fig. 1B). Tissue strain was determined by recording the displacement of 3 black markers, which were placed along the gauge length, using ImageJ [36]. Buoyancy forces are significant in this experiment, and were accounted for by subtracting the force measured with no sample present from the experimental results.

The degradation part of the force relaxation response was fit to Eq. (2) to determine the degradation rate constant, $1/\tau$ for a given instantaneous strain level [21,22]:

$$\sigma = \sigma_0 e^{-\frac{t}{\tau}} \quad (2)$$

$$\frac{1}{\tau} = -\frac{\ln\left(\frac{\sigma}{\sigma_0}\right)}{t} \quad (3)$$

where σ_0 is the relaxed nominal stress at the beginning of degradation calculated from the initial cross-sectional area. The degradation rate constant was calculated from the slope of a plot of the log of normalised stress against time, as shown in Eq. (3).

4.2. Results: Crude collagenase

A near linear increase in tissue degradation rate was found with increasing strain in circumferentially (linear fit: $R^2 = 0.912$) and axially (linear fit: $R^2 = 0.942$) strained dogbone specimens which were subjected to crude collagenase, see Fig. 5A. Lower degradation rates were observed in axial specimens compared to circumferential specimens for equivalent strain levels.

4.3. Results: Purified collagenase

To isolate the contribution of collagen in the degradation response of arterial tissue, additional stress degradation experiments were performed substituting the crude collagenase with a purified collagenase. A markedly different degradation response was found experimentally in circumferential dogbone samples treated with purified collagenase (Fig. 5B). A multi-phase response can be seen with an initial increase in the degradation rate before a region of low degradation followed by a dramatic increase.

5. Numerical modelling

5.1. The fibre-matrix unit

A theoretical model of arterial collagen degradation was developed to elucidate the complex interplay between collagenous and non-collagenous matrix components in the net mechanical response of the arterial tissue identified experimentally. This model is a one-dimensional model, named the fibre-matrix (FM) unit. A schematic of a FM unit is shown in Fig. 6A. The model consists of two elements in parallel; the left element represents the stress due to collagen fibres stretching σ_f , and the right element represents the stress in the non-collagenous matrix σ_m . The total stress, σ_{FMU} in the FM unit is given by the sum of stresses in these

Fig. 4. A) Experimental setup showing temperature controlled testing chamber and B) schematic of experimental protocol for determining degradation rate, $1/\tau$, showing the stress response to an instantaneous strain, subsequent stress relaxation for 1 h and final stress decay for a period of 3 and 7 h respectively after collagenase is added. If no collagenase is added the stress reaches an equilibrium stress σ_0 by 1 h.

Fig. 5. Degradation rate with applied strain for circumferentially (closed circles) and axially (open circles) stretched arterial dogbone samples immersed in A) crude ($n = 13$) and B) purified ($n = 16$) bacterial collagenase. Solid and dashed lines represent the linear fit for circumferential ($R^2 = 0.912$) and axial ($R^2 = 0.942$) crude experimental data respectively. Each data point represents a single sample. Note y-axis scale changes.

Fig. 6. A) Schematic showing a fibre-matrix unit which experiences a total stretch λ_t , made up of a fibre stretch λ_f and prestretch, λ_p term. The red link introduces an initial tensile or compressive strain. Only matrix stiffness k_m , and not fibre stiffness, k_f , contributes to the total stress response until the red link is fully straightened. Note: $\lambda_t \neq \lambda_f + \lambda_p$. B) Fibre-matrix units placed in parallel to make up a transmural gradient of crimp. C) Underlying V-shaped degradation response, Ψ_f for a single collagen fibre used in this study which was motivated by SALS analysis and literature [21,22]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

two elements; $\sigma_{FMU} = \sigma_f + \sigma_m$. The contribution of fibres and matrix is weighted by their volume fractions ϕ_f and ϕ_m respectively, which sum to unity.

To represent the crimping of collagen fibres that occurs in arterial tissue when there is zero internal pressure and no residual stress, a prestretch, λ_p , is introduced to the collagen fibres in the FM unit. This is achieved through a multiplicative split of the total stretch in the FM unit λ_t into a collagen fibre stretch part λ_f , and a prestretch part λ_p [28].

$$\lambda_t = \lambda_f \lambda_p \quad (4)$$

Prestretch is represented by the position of the red link in Fig. 6A and can be used to introduce either tensile or compressive strain to the collagen fibre element. For a given FM unit configuration, λ_p is a prescribed constant which does not change as load is applied. In the initial configuration $\lambda_t = 1$. Prestretch, λ_p is a measure of the degree of crimp in an individual collagen fibre. 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 from Eq. (4). It is assumed that collagen fibres do not support loads in compression, thus, the collagen fibre element contributes no stress until the chain-type link in Fig. 6A is fully straightened; i.e. $\sigma_f = 0$ until

$\lambda_f > 1$. Collagen fibres have a non-linear elastic response defined by a smoothed bilinear function with three strain domains; low, transition, and high strain [37]. The low and high strain regions are linear, while a quadratic function is used in the transition region to continuously connect the two linear regions together. The stress in a fibre is given by the continuous stepwise function,

$$\sigma_f = \phi_f \times \begin{cases} E_l \varepsilon_f, & \varepsilon_f < \varepsilon_{t1}^\sigma \\ T_1^\sigma \varepsilon_f^2 + T_2^\sigma \varepsilon_f + T_3^\sigma, & \varepsilon_{t1}^\sigma \leq \varepsilon_f \leq \varepsilon_{t2}^\sigma \\ E_h (\varepsilon_f - \varepsilon_{t2}^\sigma) + T_1^\sigma \varepsilon_{t2}^{\sigma 2} + T_2^\sigma \varepsilon_{t2}^\sigma + T_3^\sigma, & \varepsilon_f > \varepsilon_{t2}^\sigma \end{cases} \quad (5)$$

where E_l and E_h are the moduli of the low strain and high strain regions respectively, $\varepsilon_f = (\lambda_f^2 - 1)/2$ is the Green strain of the fibre, ε_{t1}^σ is the strain at which the low strain region switches to the transition region; ε_{t2}^σ is the strain at which the transition region switches to the high strain region; and the parameters T_1^σ , T_2^σ , and T_3^σ ensure continuity between the three regions and are defined as,

$$\begin{aligned} T_1^\sigma &= (E_l - E_h) / (2(\varepsilon_{t1}^\sigma - \varepsilon_{t2}^\sigma)) \\ T_2^\sigma &= E_l - \varepsilon_{t1}^\sigma (E_l - E_h) / (\varepsilon_{t1}^\sigma - \varepsilon_{t2}^\sigma) \\ T_3^\sigma &= E_l \varepsilon_{t1}^{\sigma 2} - T_1^\sigma \varepsilon_{t1}^{\sigma 2} - T_2^\sigma \varepsilon_{t1}^\sigma \end{aligned} \quad (6)$$

Table 1
Material parameters for single fibre-matrix unit calibrated from experimental data.

E_l (MPa)	E_h (MPa)	ε_{t1}^D	ε_{t2}^D	E_m (MPa)
0.004	0.4	0.3	0.7	0.02

To fully define Eq. (5) four parameters are required; E_l , E_h , ε_{t1}^D , and ε_{t2}^D .

A simple linear elastic relationship, dependent on the Green strain corresponding to λ_t , was established for the non-collagenous matrix stress; $\sigma_m = \phi_m E_m (\lambda_t^2 - 1)/2$ where E_m is the matrix stiffness. Material parameters for collagen and matrix can be found in Table 1.

5.2. Stress degradation of fibre-matrix unit

Stress degradation behaviour was modelled through reduction of the fibre and matrix volume fractions over time. This was governed by a first order rate equation,

$$\frac{d\phi_i}{dt} = -\Psi_i \phi_i \quad (7)$$

where $i = \{f, m\}$, and Ψ_i is the degradation rate constant function, which is conceptually equivalent to $1/\tau$ in Eq. (2). Eq. (7) was solved using a forward Euler method over a period of 4×10^4 s using increments of 20 s.

Importantly, motivated by our SALS structural analysis and the studies of Huang and Yannas [21] and Ghazanfari et al. [22], Ψ_f is assumed to be dependent on the collagen fibre stretch λ_f and is described with a V-shaped degradation response defined by a smoothed bilinear function, similar to that used for the collagen stress response, with three strain domains; low, transition, and high strain (Fig. 6C). The low and high strain regions are linear with negative and positive slopes respectively, while a quadratic function is used in the transition region to continuously connect the two linear regions together. The degradation rate in a fibre, Ψ_f , is then given by the continuous stepwise function,

$$\Psi_f = D_c + \begin{cases} D_l \varepsilon_f, & \varepsilon_f < \varepsilon_{t1}^D \\ T_1^D \varepsilon_f^2 + T_2^D \varepsilon_f + T_3^D, & \varepsilon_{t1}^D \leq \varepsilon_f \leq \varepsilon_{t2}^D \\ D_h (\varepsilon_f - \varepsilon_{t2}^D) + T_1^D \varepsilon_{t2}^{D2} + T_2^D \varepsilon_{t2}^D + T_3^D, & \varepsilon_f > \varepsilon_{t2}^D \end{cases} \quad (8)$$

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,

$$\begin{aligned} T_1^D &= (D_l - D_h) / (2(\varepsilon_{t1}^D - \varepsilon_{t2}^D)) \\ T_2^D &= D_l - \varepsilon_{t1}^D (D_l - D_h) / (\varepsilon_{t1}^D - \varepsilon_{t2}^D) \\ T_3^D &= D_l \varepsilon_{t1}^{D2} - T_1^D \varepsilon_{t1}^{D2} - T_2^D \varepsilon_{t1}^D \end{aligned} \quad (9)$$

To fully define Eq. (8) four parameters are required; D_l , D_h , ε_{t1}^D and ε_{t2}^D .

The matrix degradation rate constant Ψ_m is assumed to be a linear function of the total green strain,

$$\Psi_m = k_m (\lambda_t^2 - 1)/2 \quad (10)$$

where k_m is a degradation rate constant.

As the volume fractions ϕ_f and ϕ_m decrease so too do their respective stresses,

$$\sigma_f = \phi_f \sigma_f^{t=0}, \quad (11)$$

where $\sigma_f^{t=0}$ is the collagen fibre stress at time $t = 0$. The matrix stress σ_m is reduced in the same manner. Additionally, to ensure conservation of mass, degraded collagen fibre and matrix is converted into a mechanically weak component. This is simply modelled by calculating the product of the degraded volume fraction and the initial matrix stress, then multiplying this by a reduction factor R , where R is some small number which ensures its contribution is negligible. The resulting expression is,

$$\sigma_m = \phi_m \sigma_m^{t=0} + R \sigma_m^{t=0} (1 - \phi_f - \phi_m) \quad (12)$$

where $\sigma_m^{t=0}$ is the matrix stress at time $t = 0$. The expression in brackets in the second term ensures that the volume fraction equals unity.

5.3. Prestretch gradient

The ability to incorporate a prestretch λ_p into the FM unit was introduced in Section 5.1. In the zero pressure, zero residual stress, planar configuration in which experiments are conducted it is assumed that there is a gradient of collagen fibre crimp through the radial thickness of the artery wall. This transmural crimp was modelled by placing 20 FM units in parallel; each FM unit had a unique fibre prestretch. This is shown schematically in Fig. 6B.

Thus the total stress $\sigma_t = \sum_{n=1}^{20} \sigma_{FMU}^n$.

5.4. Model calibration

A collagen fibre volume fraction, ϕ_f , of 42% was used based on experimental data on collagen content for porcine arterial tissue obtained through image processing (see Supplementary data) and is supported by literature [38–40]. To model the axial experimental data, a collagen volume fraction, ϕ_f , of 5% was used which was supported by experimental data showing minimal axial collagen fibres in the porcine carotid artery [8]. This ensures that collagen fibres have a much lower contribution to the stress response of the axial test case, as in native tissue.

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.actbio.2018.06.037>.

The material properties used as an input for the model (Fig. 7) were calibrated from experimental data published previously [41]. The matrix modulus, E_m was calibrated from the initial linear stress-strain experimental response of porcine arterial tissue [39,41]. To ensure that the initial mechanical response was attributed solely to matrix, the initial response was compared to that of the vessels treated with collagenase to degrade all collagen (see Supplementary data) [41]. The bi-linear stress-strain response for collagen fibres was calibrated from the same data set as that for the matrix modulus (see Supplementary data) [41].

The V-shape degradation response was defined using the stepwise smoothed bilinear function outlined in Section 5.2. The initial negative slope, D_l , was chosen to be double that of the final positive slope, D_h , as approximately found by Huang and Yannas [21] and Ghazanfari et al. [22]. The magnitude of this function was iteratively determined from our experimental results for pure collagenase (Fig. 7). A minimum degradation rate was chosen at a strain of approximately 17% after an iterative process, corresponding closely to data from Ghazanfari et al. [22]. The constant, D_c , which simply shifts the bilinear curve along the y-axis, was set such that a minimum degradation rate irrespective of strain exists, as seen

Fig. 7. Flowchart showing the calibration process of the numerical model to capture and predict the experimental degradation behaviour.

experimentally [21,22]. This constant, D_c was chosen so that the V-shaped degradation response shifted an order of magnitude higher for the crude collagenase case, while keeping the same degradation profile, mimicking what was seen experimentally.

A reduction factor for degraded material, R , of 0.0001 was chosen through a sensitivity test, such that degraded matrix and collagen had no mechanical contribution.

The matrix degradation rate k_m was assumed to be a linear function of strain, as observed in our crude experimental results in the axial direction where it was assumed that very few collagen fibres contributed to the stress decay (Fig. 7). The matrix degradation rate k_m was subsequently calibrated from these crude collagenase results. Sensitivity to this parameter can be found in Supplementary data.

The prestretch gradient parameters, $\lambda_p^{\min}$ and $\lambda_p^{\max}$, were determined iteratively. An initial $\lambda_p^{\min}$ of 1.03 was chosen as it is the parameter that controls the strain at which degradation begins and coincided with our experimental findings. The gradient was determined iteratively to ensure we captured the degradation behaviour over a similar range to our purified collagenase results and such that all fibres are fully straightened at the start of the transition region of our stress strain curve, i.e. $\lambda_p^i > 1$ for all $i = \{1 \rightarrow n\}$. Sensitivity to the magnitude and gradient of pre-stretch can be found in the Supplementary work. The list of parameters used for degradation of the FM unit can be found in Table 2. The calibration process is described schematically in Fig. 7.

To assess the accuracy of our model, a non-parametric Spearman correlation coefficient was calculated for both the crude and purified data.

5.5. Model results: Crude collagenase

Incorporation of matrix degradation in our model allowed us to predict a similar continuously increasing degradation response to that found experimentally. The model was also capable of identifying the experimentally observed differences in degradation rate for circumferentially and axially stretched samples where fibres were parallel and perpendicular to the direction of applied strain respectively (Fig. 8A). A high correlation was seen between numerical and experimental results for both circumferential ($r = 0.940$, $p < 0.001$) and axial ($r = 0.808$, $p < 0.005$) data.

5.6. Model results: Purified collagenase

The numerical model was capable of accurately capturing the experimental response found in purified collagenase where no matrix degradation occurs. The model captured the initial degradation increase followed by a dip and subsequent increase in degradation which was observed experimentally (Fig. 8B). A high correlation ($r = 0.835$, $p < 0.001$) was also found between the experimental and numerical results for purified data.

6. Discussion

Collagen degradation is a driving force in collagen remodelling in arterial tissue, which may be critical in the progression and development of vascular disease and determining the long-term success of interventional medical devices. The goal of this study was to identify the strain dependent degradation response of, and mechanical factors influencing collagen degradation in arterial

Table 2

Model parameters calibrated and derived for crude and purified degradation models. Note that the only difference between the parameters for the crude and purified circumferential models is the magnitude of D_c and the presence of matrix degradation, k_m .

	Defined model parameters				Calibrad model parameters						
	ϕ_f	ϕ_m	$D_l \text{ ms}^{-1}$	$D_h \text{ ms}^{-1}$	$\lambda_p^{\min}$	$\lambda_p^{\max}$	c_{t1}^D	c_{t2}^D	$D_c \text{ ms}^{-1}$	$k_m (10^{-4})$	$R (10^{-4})$
Crude:											
Circumferential	0.42	0.58	−1.50	0.75	1.03	1.14	0.08	0.23	1.21	1.2	1
Axial	0.05	0.95	−1.50	0.75	1.03	1.14	0.08	0.23	1.21	1.2	1
Purified:											
Circumferential	0.42	0.58	−1.50	0.75	1.03	1.14	0.08	0.23	0.21	0	1

Fig. 8. Numerical degradation rate with applied strain for circumferentially (solid lines) and axially (dashed line) stretched arterial dogbone samples immersed in A) crude and B) purified bacterial collagenase overlaid on experimental data (circles) after calibration.

tissue, using a combination of experimental and theoretical methods.

SALS alignment analysis identified strain mediated structural changes in arterial tissue due to enzymatic degradation, suggesting that an underlying bi-linear V-shaped degradation response may exist for arterial collagen. Interestingly, mechanical testing identified two different strain mediated degradation responses in arterial tissue depending on whether crude or purified collagenase was used to degrade the tissue. A near linear degradation profile was seen experimentally for crude collagenase while a complex multi-phase response was seen in pure collagenase, both of which differed to that of pure collagen in literature [21] and what was expected from our SALS analysis. These differences identified experimentally were explained through the development of a novel 1D numerical model, however, only after a gradient of collagen fibre crimp, matrix contribution and degradation of this matrix were incorporated (see Supplementary work). Taken together, these results identify the significance of collagen fibre crimp and matrix stiffness in strain dependent arterial degradation, improving our understanding of arterial remodelling in disease development, progression and treatment.

SALS results suggest that a strain mediated degradation response exists in arterial tissue (Fig. 3). The initial increase in fibre eccentricity observed at low strain levels suggests the preferential degradation of unstrained collagen fibres, whilst the reduction in eccentricity at high strain levels suggests preferential degradation of highly strained fibres. A similar response was observed using SALS at low strain levels in corneal tissue however higher strain levels were not considered [15]. This SALS data supports the existence of a strain dependent degradation curve for collagen fibres within arterial tissue at the fibre level, even if a more complex degradation response exists at the tissue level.

Interestingly, uniaxial stress relaxation experiments using crude bacterial collagenase identified a continuous increase in degradation rate with increasing strain for circumferential and axial specimen (Fig. 5). This response differs significantly from the bi-linear V-shaped degradation response observed in reconstituted collagen tapes [21] as well as bovine pericardium [22], although it bares similarities to another study on bovine pericardium [19]. It is hypothesised that the lack of an emerging bi-linear degradation response may be due to the gradient of collagen crimp across the vessel thickness (Fig. 1A), the non-collagenous matrix mechanical contribution and in particular, the partial degradation of this matrix due to the use of crude collagenase (Fig. 1B). If true, this highlights the importance of considering collagen undulation and the role of non-collagenous constituents in

analysing collagen degradation, particularly in heterogenous tissues. The higher degradation rate in circumferential samples is believed to be associated with the higher number of load bearing collagen fibres orientated in this direction, which leads to a larger stress decay when degraded under strain. A different, more refined strain mediated degradation response was identified using purified bacterial collagenase where no matrix degradation occurs (Fig. 6). The emergent response also differs from that seen in literature with an initial increase in degradation followed by a drop off and subsequent increase in degradation rate. Interestingly, this subsequent increase was found to begin close to the transition strain (30%) of the tissue, something which has also been observed in other collagenous tissues [21,22].

The complementary numerical model helps unravel the mechanisms at play for both the crude and purified collagenase experiments. Starting with the purified case: no degradation occurs initially; as collagen fibres at low strain are crimped they bear no load and hence do not contribute to strain mediated degradation induced stress decay. Next, degradation increases as fibres begin to uncrimp and take up the load. When all fibres are fully stretched, the V-shaped behaviour resumes. To explain the crude collagenase results; matrix degradation is incorporated into the model, whereby the degradation of mechanically relevant matrix material at lower strain levels smooths the ultimate degradation response of the arterial tissue. To evaluate the degradation response in the axial direction, a collagen volume fraction of 5% was used, representing the low volume of axial fibres observed in earlier studies [8]. Interestingly, volume fractions up to 15% had minimal influence on the degradation response in the axial direction. Although a large number of parameters were required for our model, these parameters all have a physical meaning and were supported by experimental data where possible. The versatility of this model allows for the investigation of the strain dependent degradation response of other collagenous tissues by incorporating different ratios of collagen and matrix as well as different levels of collagen fibre pre-crimp (Supplementary). Increasing the collagen content and reducing the gradient of crimp results in the re-emergence of the V-shaped degradation curve of pure collagen (Fig. 6C). This may explain the strain induced protection found in tendon tissue at low strain levels [13] as well as the V-shaped degradation response observed in bovine pericardium across a larger strain range [22]. Sensitivity to these parameters can be found in Supplementary data.

The cause of the underlying V-shaped collagen degradation response, and consequently the influence on a tissue's mechanical properties, is not fully understood; however, some mechanisms

have been postulated in the literature. On the one hand, a reduction in tissue porosity with stretch has been suggested as a potential reason for an initial decrease in degradation [13,21]; while, opening up binding sites with further fibre stretching allows subsequent accelerated degradation [19,21,42]. On the other hand, conformational changes and increased monomer thermal stability due to collagen tension have also been proposed as a reason for reduced degradation [13,14,18,42,43]. Additionally, load transfer from degraded fibres to neighbouring fibres, thus increasing fibre stress has also been suggested as a potential reason for accelerated degradation [21]. The conflicting findings seen in literature may be influenced by this lack of understanding surrounding mechanisms of degradation as well as interpretation of results. For instance, it is unknown whether the degradation response differs when considering single collagen fibrils or thicker bundles of fibres. Collagen fibre damage which is likely to occur at the high strain levels experienced in this study is also likely to affect the degradation response and may influence the final degradation increase identified in this study. It is important to note that human arterial tissue has been found to be less compliant to that of porcine arterial tissue used in this study [44]. Consequently, a minimum degradation strain may exist at a lower fibre strain to that found here. Despite this difference, similar strain induced degradation trends are likely to occur in human tissue, with implications in the development and progression of disease.

One limitation of the current study is the use of bacterial collagenases to assess degradation responses to strain rather than MMPs and cysteine collagenases which are responsible for collagen degradation *in vivo* [45,46]. It is also worth noting that the effect of strain on the production of collagenase inhibitors *in vivo* has not been considered and may influence the net degradation response [45]. Additionally, the stress relaxation experiments were carried out on planar specimens and in the absence of the residual strains present within an intact vessel, although this is accounted for by incorporating a gradient of fibre crimp in our model. Finally, the presence of an intimal and adventitial layer *in vivo*, not considered in this study, may also protect the vessel from experiencing the supra physiological strain environments considered in the current study.

The results of this study have identified, for the first time, the strain dependent degradation behaviour of arterial tissue using a combination of experimental and theoretical methods. Our findings show distinctly different degradation responses when using crude or purified collagenase, highlighting the need to carefully select the appropriate enzyme when investigating tissue degradation, particularly in tests involving highly heterogeneous tissues. We have developed a 1D numerical model with an underlying V-shaped degradation response seen for pure collagen, which is capable of predicting arterial degradation through the incorporation of matrix content and a transmural gradient of collagen crimp. By incorporating matrix degradation, we were also able to explain the differences found experimentally in our crude and purified collagenase studies. These findings offer substantial new insights into arterial degradation, with implications for the design and development of mechanical devices which treat diseased arteries.

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References

- [1] A. Creane, E. Maher, S. Sultan, N. Hynes, D.J. Kelly, C. Lally, Prediction of fibre architecture and adaptation in diseased carotid bifurcations, *Biomech. Model. Mechanobiol.* 10 (2011) 831–843, <https://doi.org/10.1007/s10237-010-0277-8>.
- [2] C. Lally, F. Dolan, P.J. Prendergast, Cardiovascular stent design and vessel stresses: a finite element analysis, *J. Biomech.* 38 (2005) 1574–1581, <https://doi.org/10.1016/j.jbiomech.2004.07.022>.
- [3] A. Colombo, S. Guha, J.N. Mackle, P.A. Cahill, C. Lally, Cyclic strain amplitude dictates the growth response of vascular smooth muscle cells in vitro: role in in-stent restenosis and inhibition with a sirolimus drug-eluting stent, *Biomech. Model. Mechanobiol.* 12 (2013) 671–683, <https://doi.org/10.1007/s10237-012-0433-4>.
- [4] D.R. Nolan, C. Lally, An investigation of damage mechanisms in mechanobiological models of in-stent restenosis, *J. Comput. Sci.* 24 (2018) 132–142, <https://doi.org/10.1016/j.jocs.2017.04.009>.
- [5] A.J. Schrieffer, G. Zeindlinger, D.M. Pierce, P. Regitnig, G.A. Holzapfel, Determination of the layer-specific distributed collagen fibre orientations in human thoracic and abdominal aortas and common iliac arteries, *J. R. Soc. Interface* 9 (2012) 1275–1286, <https://doi.org/10.1098/rsif.2011.0727>.
- [6] T.C. Gasser, S. Gallinetti, X. Xing, C. Forsell, J. Swedenborg, J. Roy, Spatial orientation of collagen fibers in the abdominal aortic aneurysm's wall and its relation to wall mechanics, *Acta Biomater.* 8 (2012) 3091–3103, <https://doi.org/10.1016/j.actbio.2012.04.044>.
- [7] C. Williams, J. Liao, E.M. Joyce, B. Wang, J.B. Leach, M.S. Sacks, J.Y. Wong, Altered structural and mechanical properties in decellularized rabbit carotid arteries, *Acta Biomater.* 5 (2009) 993–1005, <https://doi.org/10.1016/j.actbio.2008.11.028>.
- [8] R.T. Gaul, D.R. Nolan, C. Lally, Collagen fibre characterisation in arterial tissue under load using SALS, *J. Mech. Behav. Biomed. Mater.* 75 (2017) 359–368, <https://doi.org/10.1016/j.jmbbm.2017.07.036>.
- [9] G.R. Douglas, A.J. Brown, J.H. Gillard, M.R. Bennett, M.P.F. Sutcliffe, Z. Teng, Impact of fiber structure on the material stability and rupture mechanisms of coronary atherosclerotic plaques, *Ann. Biomed. Eng.* 45 (2017) 1462–1474, <https://doi.org/10.1007/s10439-017-1827-3>.
- [10] L.A. Davis, S.E. Stewart, C.G. Carsten, B.A. Snyder, M.A. Sutton, S.M. Lessner, Characterization of fracture behavior of human atherosclerotic fibrous caps using a miniature single edge notched tensile test, *Acta Biomater.* 43 (2016) 101–111, <https://doi.org/10.1016/j.actbio.2016.07.027>.
- [11] H. Zahedmanesh, C. Lally, Determination of the influence of stent strut thickness using the finite element method: implications for vascular injury and in-stent restenosis, *Med. Biol. Eng. Comput.* 47 (2009) 385–393, <https://doi.org/10.1007/s11517-009-0432-5>.
- [12] S. Ghazanfari, A. Driessen-Mol, S.P. Hoerstrup, F.P.T. Baaijens, C.V.C. Bouten, Collagen matrix remodeling in stented pulmonary arteries after transapical heart valve replacement, *Cells Tissues Organs*. 201 (2016) 159–169, <https://doi.org/10.1159/000442521>.
- [13] K.E.-K. Wyatt, J.W. Bourne, P.A. Torzilli, Deformation-dependent enzyme mechanokinetic cleavage of type I collagen, *J. Biomech. Eng.* 131 (2009) 051004, <https://doi.org/10.1115/1.3078177>.
- [14] Y. Nabeshima, E.S. Grood, A. Sakurai, J.H. Herman, Uniaxial tension inhibits tendon collagen degradation by collagenase in vitro, *J. Orthop. Res.* 14 (1996) 123–130, <https://doi.org/10.1002/jor.1100140120>.
- [15] M.C. Robitaille, R. Zareian, C.A. Dimarzio, K.-T. Wan, J.W. Ruberti, Small-angle light scattering to detect strain-directed collagen degradation in native tissue, *Interface Focus* 1 (2011) 767–776, <https://doi.org/10.1098/rsfs.2011.0039>.
- [16] B.P. Flynn, A.P. Bhole, N. Saeidi, M. Liles, C.A. DiMarzio, J.W. Ruberti, Mechanical strain stabilizes reconstituted collagen fibrils against enzymatic degradation by mammalian collagenase matrix metalloproteinase 8 (MMP-8), *PLoS ONE* 5 (2010), <https://doi.org/10.1371/journal.pone.0012337> e12337.
- [17] J.W. Ruberti, N.J. Hallab, Strain-controlled enzymatic cleavage of collagen in loaded matrix, *Biochem. Biophys. Res. Commun.* 336 (2005) 483–489, <https://doi.org/10.1016/j.bbrc.2005.08.128>.
- [18] A.P. Bhole, B.P. Flynn, M. Liles, N. Saeidi, C.A. Dimarzio, J.W. Ruberti, Mechanical strain enhances survivability of collagen microneurites in the presence of collagenase: implications for load-bearing matrix growth and stability, *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 367 (2009) 3339–3362, <https://doi.org/10.1098/rsta.2009.0093>.
- [19] J.C. Ellsmere, R.A. Khanna, J. Michael Lee, Mechanical loading of bovine pericardium accelerates enzymatic degradation, *Biomaterials*. 20 (1999) 1143–1150, [https://doi.org/10.1016/S0142-9612\(99\)00013-7](https://doi.org/10.1016/S0142-9612(99)00013-7).
- [20] A.S. Adhikari, E. Glassey, A.R. Dunn, Conformational dynamics accompanying the proteolytic degradation of trimeric collagen I by collagenases, *J. Am. Chem. Soc.* 134 (2012) 13259–13265, <https://doi.org/10.1021/ja212170b>.
- [21] C. Huang, I.V. Yannas, Mechanochemical studies of enzymatic degradation of insoluble collagen fibers, *J. Biomed. Mater. Res.* 11 (1977) 137–154, <https://doi.org/10.1002/jbm.820110113>.
- [22] S. Ghazanfari, A. Driessen-Mol, C.V.C. Bouten, F.P.T. Baaijens, Modulation of collagen fiber orientation by strain-controlled enzymatic degradation, *Acta Biomater.* 35 (2016) 118–126, <https://doi.org/10.1016/j.actbio.2016.02.033>.
- [23] R. Rezakhaniha, A. Agianniotis, J.T.C. Schrauwen, A. Griffa, D. Sage, C.V.C. Bouten, F.N. Van De Vosse, M. Unser, N. Stergiopulos, Experimental investigation of collagen waviness and orientation in the arterial adventitia

- using confocal laser scanning microscopy, *Biomech. Model. Mechanobiol.* 11 (2012) 461–473, <https://doi.org/10.1007/s10237-011-0325-z>.
- [24] J.T.C. Schrauwen, A. Vilanova, R. Rezakhanlou, N. Stergiopoulos, F.N. van de Vosse, P.H.M. Bovendeerd, A method for the quantification of the pressure dependent 3D collagen configuration in the arterial adventitia, *J. Struct. Biol.* 180 (2012) 335–342, <https://doi.org/10.1016/j.jsb.2012.06.007>.
- [25] W. Krasny, C. Morin, H. Magoaric, S. Avril, A comprehensive study of layer-specific morphological changes in the microstructure of carotid arteries under uniaxial load, *Acta Biomater.* 57 (2017) 342–351, <https://doi.org/10.1016/j.actbio.2017.04.033>.
- [26] I. Hariton, G. DeBotton, T.C. Gasser, G.A. Holzapfel, Stress-driven collagen fiber remodeling in arterial walls, *Biomech. Model. Mechanobiol.* 6 (2007) 163–175, <https://doi.org/10.1007/s10237-006-0049-7>.
- [27] S. Loerakker, T. Ristori, F.P.T. Baaijens, A computational analysis of cell-mediated compaction and collagen remodeling in tissue-engineered heart valves, *J. Mech. Behav. Biomed. Mater.* 58 (2016) 173–187, <https://doi.org/10.1016/j.jmbbm.2015.10.001>.
- [28] S. Loerakker, C. Obbink-Huizer, F.P.T. Baaijens, A physically motivated constitutive model for cell-mediated compaction and collagen remodeling in soft tissues, *Biomech. Model. Mechanobiol.* 13 (2013) 985–1001, <https://doi.org/10.1007/s10237-013-0549-1>.
- [29] T.A.M. Heck, W. Wilson, J. Foolen, A.C. Cilingir, K. Ito, C.C. van Donkelaar, A tissue adaptation model based on strain-dependent collagen degradation and contact-guided cell traction, *J. Biomech.* 48 (2015) 823–831, <https://doi.org/10.1016/j.jbiomech.2014.12.023>.
- [30] T.K. Tonge, J.W. Ruberti, T.D. Nguyen, Micromechanical modeling study of mechanical inhibition of enzymatic degradation of collagen tissues, *Biophys. J.* 109 (2015) 2689–2700, <https://doi.org/10.1016/j.bpj.2015.10.051>.
- [31] N.J. Driessen, R.A. Boerboom, J.M. Huyghe, C.V. Bouten, F.P. Baaijens, Computational analyses of mechanically induced collagen fiber remodeling in the aortic heart valve, *J. Biomech. Eng.* 125 (2003) 549–557, <https://doi.org/10.1115/1.1590361>.
- [32] E. Müller-Schweinitzer, Cryopreservation of vascular tissues, *Organogenesis* 5 (2009) 97–104, <https://doi.org/10.4161/org.5.3.9495>.
- [33] D.E. Pegg, M.C. Wusteman, S. Boylan, Fractures in cryopreserved elastic arteries, *Cryobiology* 34 (1997) 183–192, <https://doi.org/10.1006/cryo.1996.1997>.
- [34] A.J. Schriefel, T. Schmidt, D. Balzani, G. Sommer, G.A. Holzapfel, Selective enzymatic removal of elastin and collagen from human abdominal aortas: uniaxial mechanical response and constitutive modeling, *Acta Biomater.* 17 (2015) 125–136, <https://doi.org/10.1016/j.actbio.2015.01.003>.
- [35] G.A. Holzapfel, G. Sommer, M. Auer, P. Regitnig, R.W. Ogden, Layer-specific 3D residual deformations of human aortas with non-atherosclerotic intimal thickening, *Ann. Biomed. Eng.* 35 (2007) 530–545, <https://doi.org/10.1007/s10439-006-9252-z>.
- [36] C. a Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, *Nat. Methods* 9 (2012) 671–675, doi:10.1038/nmeth.2089.
- [37] C.A. O'Connor, D.R. Nolan, E. McEvoy, J.P. McGarry, A three part hyperelastic law for anisotropic aortic tissue: Model development and experimental validation, in: Summer Biomech. Bioeng. Biotransport Conf., Arizona, 2017.
- [38] A. Tsamis, J.T. Krawiec, D.A. Vorp, Elastin and collagen fibre microstructure of the human aorta in ageing and disease: a review, *J. R. Soc. Interface* 10 (2013) 20121004, <https://doi.org/10.1098/rsif.2012.1004>.
- [39] L. de Figueiredo, R.G. Borges, R.R. Jaldin, N.A.G. Dias, J.B. Stolf, P.S. Michel, Gutierrez, collagen is reduced and disrupted in human aneurysms and dissections of ascending aorta, *Hum. Pathol.* 39 (2008) 437–443, <https://doi.org/10.1016/j.humpath.2007.08.003>.
- [40] G.M. Fischer, J.G. Llauro, Collagen and elastin content in canine arteries selected from functionally different vascular beds, *Circ. Res.* 19 (1966) 394–399, <https://doi.org/10.1161/01.RES.19.2.394>.
- [41] M. Ghasemi, D.R. Nolan, C. Lally, A constituent specific study of damage accumulation in arterial Tissue, *Biomech. Model. Mechanobiol.* (2018). Accepted.
- [42] S.-W. Chang, M.J. Buehler, Molecular biomechanics of collagen molecules, *Mater. Today* 17 (2014) 70–76, <https://doi.org/10.1016/j.mattod.2014.01.019>.
- [43] C.A. Miles, M. Ghelashvili, Polymer-in-a-box mechanism for the thermal stabilization of collagen molecules in fibers, *Biophys. J.* 76 (1999) 3243–3252, [https://doi.org/10.1016/S0006-3495\(99\)77476-X](https://doi.org/10.1016/S0006-3495(99)77476-X).
- [44] G. Sommer, G.A. Holzapfel, 3D constitutive modeling of the biaxial mechanical response of intact and layer-dissected human carotid arteries, *J. Mech. Behav. Biomed. Mater.* 5 (2012) 116–128, <https://doi.org/10.1016/j.jmbbm.2011.08.013>.
- [45] H. Abdul-Hussien, R.G.V. Soekhoe, E. Weber, J.H. von der Thüsen, R. Kleemann, A. Mulder, J.H. van Bockel, R. Hanemaaijer, J.H.N. Lindeman, Collagen degradation in the abdominal aneurysm, *Am. J. Pathol.* 170 (2007) 809–817, <https://doi.org/10.2353/ajpath.2007.060522>.
- [46] M. Aikawa, Vascular biology of collagenases in vulnerable atherosclerotic plaques, *Int. Congr. Ser.* 1262 (2004) 67–70, <https://doi.org/10.1016/j.ics.2003.12.056>.