Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

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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ARTICLE INFO

Article history:
Received 27 March 2018
Received in revised form 4 June 2018
Accepted 29 June 2018
Available online xxxx

Keywords:
Collagen fibre degradation
Strain dependent degradation
Arterial tissue
Collagenase
Numerical model

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, heterogenous 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.

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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https://doi.org/10.1016/j.actbio.2018.06.037
1742-7061/

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, Robitialle 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 heterogenous 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°C/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 ± SEM (SD).

Please cite this article in press as: R.T. Gaul et al., Strain mediated enzymatic degradation of arterial tissue: Insights into the role of the non-collagenous tissue matrix and collagen crimp, Acta Biomater. (2018), https://doi.org/10.1016/j.actbio.2018.06.037