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STRAIN MEDIATED ENZYME DEGRADATION OF ARTERIAL TISSUE; IMPLICATIONS IN DISEASE AND MEDICAL DEVICE DESIGN

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INTRODUCTION

Collagen fibre architecture is of critical importance in healthy arterial function. It is believed that maladaptive remodelling of this architecture may play a role in the development and progression of arterial disease [1]. To date, literature has focused on load induced fibre reorganisation with a clear lack of information available on collagen production and degradation in response to load in arterial tissue.

Previous studies on other soft tissues, such as tendon and bovine pericardium (BP), have shown conflicting results on whether load inhibits [2] or enhances [3] enzymatic activity. In addition, dynamic loading has been shown to exhibit an accelerated enzymatic response in BP [3]. It is hypothesised that mechanical loading may also influence the enzymatic activity of collagenase in arteries, although further investigation is needed to decipher these mechanisms.

Small angle light scattering (SALS) is a technique which lends itself well to investigating load induced degradation as it allows structural information to be acquired non-destructively for fibrous test specimens. In fact, SALS has previously been used to identify the preferential degradation of unloaded collagen fibres in collagenase treated corneal tissue in real time [4].

The aim of the present study is to use a SALS system to measure collagen fibre changes in intact arterial tissue and to explore the influence of load on collagen degradation in arterial tissue.

METHODS

Porcine common carotid arteries were dissected into 5 mm ring samples before being cut longitudinally to obtain planar samples. The adventitial layer was carefully removed prior to testing to allow adequate light transmission for future SALS analysis. Samples were placed in a custom stretching device at 0%, 5% and 25% circumferential strain levels, representative of stiff, healthy and weakened tissue respectively. Strained samples were incubated in bacterial collagenase (Clostridium histolyticum, Sigma Aldrich) at 37°C.

Samples were analysed at 1-hour intervals for a total of 4 hours. Measurements were taken at the same 64 interrogation regions at each time point. Testing was carried out using an in-house SALS system consisting of a 5mW HeNe laser (λ = 632.8 nm) and single focusing lens (f_l = 75 mm). Each sample was interrogated sequentially using a 150 µm beam diameter and a 250 µm scanning resolution, controlled using LabVIEW.

Fibre alignment was determined based on the eccentricity of the scattered light distribution using Eq. 1. Histological analysis was also carried out on the same samples post collagenase treatment to support SALS data.

$$E = 2\frac{\sqrt{\left(\frac{Major\ Axis}{2}\right)^2 - \left(\frac{Minor\ Axis}{2}\right)^2}}{Major\ Axis} \quad (1)$$

RESULTS

SALS images of a sample exposed to 5% strain and collagenase for 4 hours showed increased fibre alignment based on scattered light eccentricity, see Figure 1a) and 1b). Figure 1c) shows the relative change in SALS eccentricity under different loading conditions in the presence of collagenase for 4 hours (Two outliers have been removed based on the ROUT method [5]). The relative change in fibre alignment is also shown at each time point for a single sample at 5% and 25% strain in Figure 1d). Whilst variations exist in the initial fibre angle and the degree of fibre eccentricity, the change in fibre eccentricity is measured relative to the initial fibre eccentricity of each sample prior to collagenase treatment.

Histological images of the same samples also show contrasting fibre organisation based on the loading conditions applied to the vessel wall, see Figure 2.

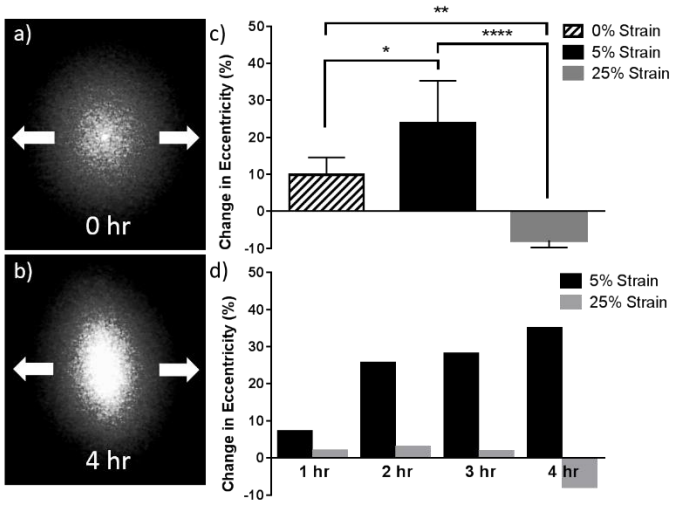

Figure 1: a) and b) SALS light distribution after 4 hours at 5% strain subjected to collagenase. Relative change in SALS eccentricity in the presence of collagenase c) after 4 hours and d) at each time point. N=7, * P ≤ 0.05, ** P ≤ 0.01, **** P ≤ 0.0001

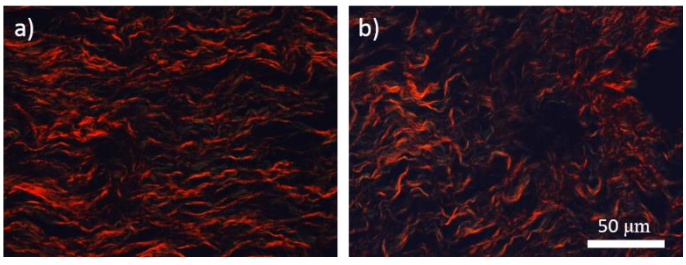

Figure 2: Polarised light images of picrosirius red stained arterial tissue showing collagen fibre organisation in arterial tissue subjected to a) 5% strain and b) 25% strain.

DISCUSSION

Results shown in Figure 1 demonstrate that strain mediated degradation mechanisms exist in arterial tissue which are dependent on the level of strain experienced by the tissue.

The large relative increase in SALS eccentricity at 5% strain indicates increased fibre alignment over time and therefore preferential degradation of unloaded fibres. In contrast, a 25% strain resulted in a reduction in eccentricity suggesting less fibre alignment over time and consequently, the preferential degradation of loaded fibres. The relatively small increase in alignment in the 0% strain condition was attributed to a greater signal to noise ratio as sample density decreases over time with a more isotropic collagen degradation. These findings are supported by histological analysis where a clear lack of collagen organisation is evident in tissue subjected to 25% strain (Figure 2).

In earlier published work by Huang and Yannes [6], a similar degradation response was identified in reconstituted bovine collagen, in the form of a tape. A protective mechanism was identified at low strain levels (< 4%) whilst accelerated collagen degradation was observed at higher strain levels (> 6%) [5]. This study demonstrates that collagen under load within arterial tissue responds to collagen degrading enzymes in a similar fashion. This load–mediated degradation process may have implications in disease where weakening of the vessel wall could result in higher strains which induce further collagen degradation through a positive feedback mechanism.

Future work aims to explore a range of different loading regimes, including physiological and diseased conditions, to more fully investigate load mediated collagen degradation and production in response to mechanical stimuli. These results not only provide important information on the development and progression of arterial disease, but also its treatment, where the mechanical environment may be artificially altered, e.g. stenting.

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