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Measurement of Friction-induced Changes in Pig Aorta Fibre Organization by Non-invasive Imaging as a Model for Detecting the Tissue Response to Endovascular Catheters

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ABSTRACT

Alterations in quantity or architecture of elastin and collagen fibres are associated with some blood vessel pathologies. Also some medical interventions such as endovascular catheterization have the potential to damage blood vessels. This study reports the use of porcine aorta as a model system for studying the physical impact of catheters on vasculature, in conjunction with non-invasive imaging techniques to analyse collagen and elastin fibre organization and assess load-induced changes. Porcine aorta was exposed to frictional trauma and elastin and collagen fibre orientation evaluated by destructive, histochemical methods and non-invasive imaging. The latter allowed the immediate impact of force on fibre orientation and fibre recovery to be evaluated longitudinally.

In normal aorta, elastin fibres are aligned at the surface, but become less aligned with increasing depth, showing no alignment by ~30 μm. Collagen fibres meanwhile appear aligned down to a depth of 35 μm. Changes in collagen and elastin fibre orientation in healthy pig aorta were detected by conventional destructive histology within 5 min of application of a sliding 10 N load, while lesser loads had less impact. Good recovery of fibre orientation was observed within 20 min. Non-invasive imaging of *ex vivo* aorta tissue provides a good indication of the extent of fibre re-organization following frictional stress, at loads similar to those encountered during medical interventions such as catheterization. These results indicate that tissue deformation can occur from these procedures, even in healthy tissue, and highlight the potential for the development of an *in vivo* probe capable of monitoring vascular changes in patients.

1. Introduction

Vascular surgeons increasingly use sophisticated endovascular catheters to investigate and treat vascular complications (*e.g.*, ablation catheters [1], and force controlled and haptic catheters [2–4]). In the hands of experienced surgeons, perforations of blood vessels with endovascular catheters are very rare, however many of the patients being treated potentially have diseased vessels; in these cases even experienced surgeons may find that the use of the endovascular catheters leads to some damage of blood vessels. The currently available catheter systems are sometimes difficult and slow to manipulate into position, thereby, reducing their ability to safely interact with the blood vessels insertion and decrease the likelihood of blood vessel damage. However, a reduction in friction also decreases the degree of haptic feedback, which provides the surgeon with valuable information about the progress of the procedure. In addition, the insertion of a catheter can increase the risk of platelet aggregation in blood flow [5], but, in most cases, the patient is provided with an appropriate dosage of heparin. Damage to the extracellular matrix (ECM) will require a wound healing response and if it goes wrong this can lead to fibrosis [6]. Thus manoeuvrability represents a critical design feature for these catheters [7]. This feature is highly dependent on minimising the coefficient of

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friction between the catheter and the blood vessels. In response to a frictional insult, fibre production and orientation can change. This is why it is important to look at both when assessing damage inflicted by rubbing a catheter along aortic tissue. A problem in improving endovascular catheter design is obtaining information on the extent of the damage to blood vessels which may occur when catheters are used. The vascular endothelial surface layer (ESL) is notoriously fragile and may undergo damage during catheterization and repair post catheterization. ESL assessment has not proved a useful biological indicator of catheter–induced damage. This is because in almost all cases, patients undergoing catheterization as a result of cardiovascular disease may already have pre-existing damage to their glycocalyx. In addition, the endothelium of such patients is potentially more vulnerable to damage by the catheter [8].

Accordingly the approach taken in this study was to look beneath the ESL and examine the relationship between mechanical stress caused by friction (using materials used in endovascular catheter tips) and the deformation of collagen and elastin fibres in pig aorta tissue *ex vivo*.

Our aims in this study were to use an experimental system to apply loads similar to those occurring during endovascular catheterization; to examine whether there was a predictable change in collagen and elastin fibre organization related to the level of frictional stress applied; to examine the extent to which fibre deformation was permanent or recoverable and; most importantly, to explore the possibility of using non-invasive imaging technology to evaluate collagen content and fibre reorientation after frictional insult. The strength of the aorta depends on the organization of fibres in the ECM. In the aorta the adventitial layer contains mainly collagen, while the medial layer consists of collagen and elastin [9]. The orientation of collagen fibres is important in providing tensile strength while the elastin provides elasticity to tissues, allowing them to return to their original state post deformation [10].

In this study we first examined the effect of physical insult on collagen and elastin fibres in pig aorta by fixing the tissue post insult and characterizing the fibre orientation by haematoxylin and eosin staining. We then developed a holder which kept the aorta at a pre-stretched load and allowed the application of a single pass at a predetermined load, of a 4 mm diameter sphere of PEBA (polyether block amide), commonly used in the manufacture of endovascular catheter tips. We used second harmonic generation (SHG) imaging and two photon excitation fluorescence (TPEF) to detect changes in the organization of the collagen and elastin fibres, respectively.

2. Materials and Methods

All chemicals were obtained from Sigma-Aldrich (Dorset, UK) unless otherwise stated.

2.1. Pig aorta Preparation

Porcine hearts with aortas attached were purchased from a local butcher 24 h after slaughter and stored in phosphate buffered saline (PBS, Oxoid, Hampshire, UK) at 4 °C for transportation, which generally took less than 1 h. On arrival at the laboratory, the aortas were immediately detached from the hearts with a scalpel and washed three times in sterile PBS. Pieces of aorta ~4 cm long were cut along the main axis to obtain rectangular, flat strips of aortic tissue, which could be stretched longitudinally.

On examination we found the retention of endothelial cells was very patchy and uneven – almost certainly due to trauma during handling (confirmed by silver nitrate staining for the endothelium – results not shown). Since we were unable to obtain fresh samples with intact endothelia and ESL assessment has not proved useful in the determination of catheter–induced damage [8], we focused on studying the impact of force on the collagen and elastin fibres of the aorta.

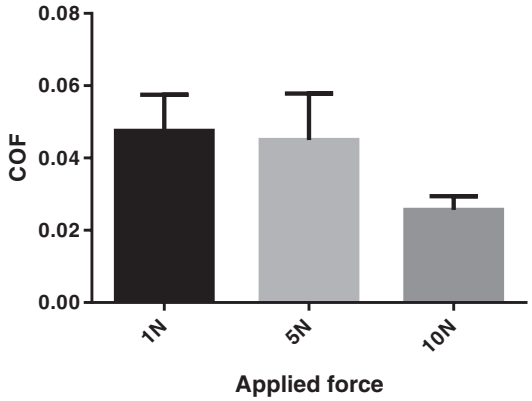

Fig. 1. Average friction behaviour of PEBA ball.

2.2. Friction Test

Friction tests were carried out using a CETR-UMT 2 (Bruker, Massachusetts, USA) tribometer with the load stabilized for 20 s before testing. The friction test samples were immersed in a blood substitute solution (0.9 mM dextran 70, 155 mM NaCl) to replicate blood viscosity and rheological behaviour [11]. To mimic the *in vivo* state of the unpressurised aorta, a tissue sample holder was developed by Philips Research, Eindhoven, Netherlands and the West Pomeranian University of Technology, Szczecin, Poland to hold the aorta sample during the friction test and maintain uniaxial tension. This device has been described elsewhere [12]. The fresh pig aorta was flattened in the longitudinal direction, the sample length increased by 15%, reflecting the degree of stretching observed during normal blood flow, and the sample held in place.

In the preliminary tests a catheter tip, made of a polymer matrix including PEBA (Boston Scientific 6F Guide Catheter (Massachusetts, USA) with 2.1 mm external diameter, 1.7 mm internal diameter) with a 'hollow tube' tip geometry, was held at 45° with respect to the friction surface during sliding. Normal loads in the range of 100 mN - 3 N were applied as a single pass along the longitudinal axis of the aorta, the samples were fixed within 10 min of the frictional insult, sectioned and haematoxylin and eosin (H & E) stained. Tests were also carried out with a 4 mm diameter steel ball at 5 N.

Subsequent tests used an injection-moulded 4 mm diameter PEBA ball (Pebax® 3533, hardness ShD 33, courtesy of Philips Research, Eindhoven, Netherlands), held perpendicular to the plane of the tissue and driven once across the sample along the longitudinal axis, at 1 mm/s, with 1, 5 or 10 N applied normal load. Initially, the fibre response was analysed as before by fixing the tissue immediately after frictional insult. In addition some samples were allowed to rest for 90 min prior to fixing to evaluate fibre recovery. In all cases these samples were H & E stained.

In the final experiments, the fixing and staining procedure was replaced by non-invasive imaging of the samples with a confocal microscope since this facilitated repeated imaging of the same area during the recovery process.

2.3. Preparation of Histological Sections

The samples were fixed in 10% formaldehyde solution, processed and embedded in paraffin blocks. Three 5 µm thick sections were cut from each block and stained with haematoxylin and eosin.

2.4. Confocal Imaging

Collagen was visualized within the samples by SHG imaging in the epi-direction at different depths using a Zeiss LSM 510 Meta upright laser-scanning confocal microscope (Oberkochen, Germany) attached