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Mechanical, biological and structural characterization of human atherosclerotic femoral plaque tissue

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ABSTRACT

The failure of endovascular treatments of peripheral arterial disease represents a critical clinical issue. Specialized data are required to tailor such procedures to account for the mechanical response of the diseased femoral arterial tissue to medical device deployment. The purpose of this study is to characterize the mechanical response of atherosclerotic femoral arterial tissue to large deformation, the conditions typical of angioplasty and stenting, and also to determine the mechanically induced failure properties and to relate this behaviour to biological content and structural composition using uniaxial testing, Fourier transform infrared spectroscopy and scanning electron microscopy. Mechanical and biological characterization of 20 plaque samples obtained from femoral endarterectomy identified three distinct classifications. "Lightly calcified" samples display linear mechanical responses and fail at relatively high stretch. "Moderately calcified" samples undergo an increase in stiffness and ultimate strength coupled with a decrease in ductility. Structural characterization reveals calcified nodules within this group that may be acting to reinforce the tissue matrix, thus increasing the stiffness and ultimate strength. "Heavily calcified" samples account for the majority of samples tested and exhibit significantly reduced ultimate strength and ductility compared to the preceding groups. Structural characterization of this group reveals large areas of calcified tissue dominating the failure cross-sections of the samples. The frequency and structural dominance of these features solely within this group offers an explanation as to the reduced ultimate strength and ductility and highlights the need for modern peripheral endovascular devices to account for this behaviour during novel medical device design.

1. Introduction

Atherosclerosis is a chronic inflammatory response of the arterial wall to lipid accumulation beneath the endothelium. Its progression is characterized by monocyte infiltration, lipid oxidation, foam cell formation, smooth muscle cell migration [1,2] and the gradual deposition of cholesterol crystals, cellular waste products and calcium minerals [3,4]. The continued deposition of calcium minerals can lead to atherosclerotic plaques that contain large calcified lesions. Such heavily calcified plaques are viewed as a common feature of advanced atherosclerosis and are regarded as the most established atheroma in the arterial tree [5–7].

The manifestation of the atherosclerotic process within the femoral arteries is known as lower extremity peripheral arterial disease (PAD). The narrowing of the femoral vessels that accompanies this disease process causes a myriad of conditions such as claudication and ischaemia, which are associated with high morbidity, mortality and the impairment of quality of life [8]. The endovascular treatment of atherosclerotic femoral vessels to alleviate this condition has been established as a safe and effective method of combating PAD [9–11]. However, despite the proven efficacy of minimally invasive femoral revascularization, the implementation of techniques such as angioplasty, stenting and drug delivering therapies such as drug-eluting stents and drug-coated balloons, is still impeded by numerous complications. The main issue regarding these treatments is the development of restenosis [12–14]; however; the issues of arterial dissection [15,16] and stent fracture [17–19] also hinder the progress of this treatment modality.

The failure of endovascular treatments of PAD therefore represents a critical clinical issue. From a medical device design perspective, specialized experimental data are required in order to tailor such therapies to account for the mechanical response of femoral atherosclerotic tissue during balloon and stent deployment and also to facilitate the development of accurate material models to

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represent the diseased portion of atherosclerotic femoral vessels during numerical simulations of novel device designs. However, there is a paucity of data regarding the mechanical behaviour of femoral plaque tissue. Of the studies that do characterize the mechanical behaviour of such tissue [20,21], none examine the mechanically induced failure properties, the mechanical response of the tissue to large deformation, or the effect of biological content and structural composition on overall mechanical behaviour. These characteristics are of vital importance when considering the effect of medical device deployment on plaque mechanics as such procedures expose plaques of varying composition to deformations significantly larger than those experienced within the in vivo physiological strain range. These procedures also induce plaque failure through processes distinct from the mechanisms that trigger spontaneous fatigue failure of vulnerable plaques, which is believed to occur through a combination of fibrous cap thinning and transient lesion collapse [22,23]. This differs from mechanically induced failure which occurs due to circumferential stresses induced on the plaque structure due to medical device deployment [24]. Two distinct types of plaque failure therefore exist and this study seeks to characterize the mechanically induced failure properties of the diseased tissue so as to develop specialized data regarding the mechanical response of femoral plaque tissue to medical device deployment.

In order to characterize this behaviour, the mechanical response of human femoral plaque tissue is examined using uniaxial testing and then related to plaque biological composition as determined using Fourier transform infrared (FTIR) spectroscopy. This study also employs scanning electron microscopy (SEM) to structurally characterize the failure cross-sections of plaque samples after uniaxial testing in order to relate structural features to mechanical behaviour. This study therefore adds to the limited knowledge base regarding the mechanical behaviour of plaques originating in the femoral arteries and also relates this behaviour to the biological content and structural composition of the diseased tissue.

2. Materials and methods

2.1. Sample acquisition

Femoral plaques from 15 patients were obtained from the Limerick University Hospital, Limerick, Ireland in a manner that conformed to the Declaration of Helsinki and was approved by the hospital's Ethical Research Committee. The femoral plaques were collected from consenting patients who underwent femoral endarterectomy during femoral-popliteal bypass surgery to treat lower extremity PAD. Within this population, 80% (12/15) of the patients were male, with a median age of 73.25 years (range 60–81) and the median age of the female population was 78.67 years (range 73–82). Plaques were surgically removed in toto from the femoral artery with preservation of plaque structural integrity emphasized to minimise possible disruption of the plaque luminal surface (Fig. 1).

The plaque samples were frozen in phosphate buffer solution (PBS) immediately after removal at −20 °C. On the day of tissue testing, each plaque was equilibrated to room temperature in PBS and further heated to 37 °C prior to FTIR analysis, mechanical testing and SEM examination. Each plaque underwent the process illustrated in Fig. 2.

Pre-operative patient data and the ankle brachial pressure index (ABPI) were recorded at the time of sample extraction (Table 1). The ABPI is calculated by dividing the systolic blood pressure measured in the arteries at ankle level by the systolic blood pressure measured in the brachial artery. It is used to assess patients for the presence and severity of PAD as a fall in blood pressure in an artery at ankle level relative to the central blood pressure would suggest a stenosis in the arteries at a location between the aorta and the ankle [25]. The clinical interpretation of the ABPI values listed in Table 1 is available in Ref. [26].

2.2. Mechanical testing

Uniaxial mechanical testing was carried out on 20 plaque samples obtained from the 15 patients using a uniaxial tester and video extensometer developed in-house. Samples were elongated in the circumferential direction in order to determine the mechanical response of each sample to large deformation and the point of mechanically induced plaque failure. Plaque 1 was divided into three separate pieces during surgical removal and each piece was sufficiently large to be tested individually according to the dimensions outlined by Mulvihill and Walsh [27]. Plaques 4, 6 and 7 were divided into two separate pieces during the surgical removal and again each piece was sufficiently large to be tested individually. This increased the sample size to $n = 20$ (i.e. plaques 1a, 1b, 1c, 4a, 4b, 6a, 6b, 7a and 7b).

Samples were secured within the testing apparatus using clamps designed for soft biological tissue and a uniform torque of 50 cN m was applied to the clamps using a torque screwdriver [28]. Measurements of the gauge length and width were taken using a vernier calipers and also with a non-contact photography system to validate the values. The thickness was measured using a thickness gauge and again with a non-contact photography system to validate the values.

Samples were preconditioned using 5 cycles to 10% stretch at a displacement rate of 0.1 mm s^{-1} and then elongated to failure at a displacement rate of 30% of gauge length per second [29,30]. The plaques typically had width-to-length ratios greater than 4:1 which indicates that the samples are in planar shear during testing [27]. It should be noted that this mode of testing differs from tensile testing regarding the assumptions that are made about the minor stretch components. These components effect the characterization of the first strain invariant which is necessary to develop a strain energy function to model the behaviour of the mechanical data generated in this study. Future studies wishing to numerically model the results generated by this work should be conscious of the fact that the samples were tested in planar shear.

2.3. FTIR analysis

FTIR analysis (Spectrum 100, Perkin Elmer Inc., MA) was performed over the plaque luminal surface using attenuated total reflectance (ATR) to characterize the global biological content of the samples. A background spectrum was initially removed and the ATR diamond crystal was placed in direct contact with the

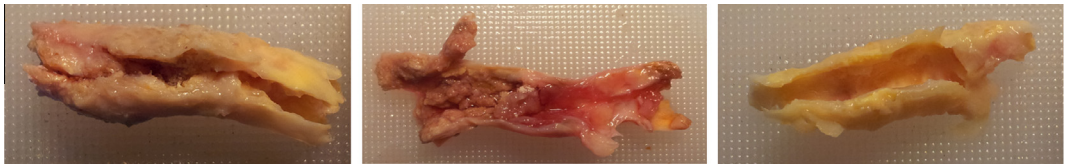

Fig. 1. Femoral plaque samples post-endarterectomy.

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